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ASH COMPOSITION OF UPLAND RICE AT VARIOUS STAGES OF GROWTH

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INTRODUCTION

The following ash analyses of upland rice (*Oryza sativa*) at various stages of growth were made in connection with a study of the effect of lime-induced chlorosis on the ash composition of the plant. In the course of this work it was necessary to know particularly how the iron content of the plant varied with its age. The analyses are reported here, as it is believed that such data are of general importance in explaining certain peculiarities of crop growth.

Kelley and Thompson [1] have already investigated the composition of rice at different stages of growth, but their study did not suffice for our purpose, as it covered only the last half or third of the growing period and did not include iron and some other ash constituents.

EXPERIMENTAL METHODS EMPLOYED

The plants were grown in large cylinders sunk in the ground and protected by wire netting (4 meshes to the inch). Each cylinder afforded a surface of 7 square feet of soil in which 29 plants were grown.

Porto Rican red-clay soil, which is well adapted for rice, was used in the cylinders. This was fertilized liberally with sulphate of ammonia, acid phosphate, and muriate of potash, so that the ash composition might not be influenced at any stage by a lack of nutrients. Fertilizers furnishing 10 gm. of nitrogen (N), 5 gm. of phosphoric acid (P_2O_5) and 10 gm. of potash (K_2O) were incorporated with the soil before planting; when the plants were 18 days old, a surface application of 2 gm. of nitrogen, 1 gm. of phosphoric acid, and 2 gm. of potash was made; and when the plants were 59 days old, 3 gm. of nitrogen, 3 gm. of phosphoric acid, and 3 gm. of potash were applied.

[1] Kelley, W. P., and Thompson, Alice R. A study of the composition of the rice plant. Hawaii Agr. Exp. Sta. Bul. 21, 51 p. 1910.

The plants were watered only a few times, during an occasional dry spell, and made an excellent growth. The growing period was from June 15 to October 16, during which time the weather conditions were fairly uniform, with high temperature and humidity. The average monthly mean temperature ranged from 77.2° in June to 79° F. in October. The monthly precipitations from June to September were, respectively, 10.90, 11.98, 11.67, and 8.22 inches. There were, however, some dry spells of a week or 10 days that apparently affected the plants; note of this is made below.

At 18 days the plants were thinned from 40 to 29 in each cylinder, at which number they were kept during growth. The 11 plants removed from each cylinder at this time served for the 18-day-old sample, while for the 26-day-old sample 6 cylinders were cut; for the 48-day-old sample, 5 cylinders; and for the succeeding samples, 4 cylinders each. As it was impossible to remove the roots completely from the heavy clay soil, the weight of the roots is not recorded. The roots were removed, however, as completely as possible for analysis.

In preparing the samples for analysis each leaf and stalk was washed individually immediately after cutting to guard against loss of mineral matter by leaching. Under such conditions there was probably a certain loss of mineral matter from withered leaves, but no appreciable loss from the green leaves. However, this is practically of little importance, as the conditions of washing, while thorough, were no more severe than those to which the plant would be subjected by rainfall. Even digesting the leaves in cold water for 15 minutes extracted little mineral matter from green leaves. Forty-five gm. of green rice leaves previously washed on the plant were stirred up with 1 liter of distilled water. The water on evaporation yielded a residue of 0.008 gm. of mineral matter, part of which was due to minute leaf hairs broken off in the stirring; 9 gm. of withered leaves soaked for 15 minutes in 500 c. c. of water left a residue of 0.057 gm. of mineral matter.

The analytical methods employed were essentially those of the Association of Official Agricultural Chemists,[1] with a few exceptions. Preparation of the ash was by the optional method, igniting over a very low flame without calcium acetate and leaching when necessary. Iron was determined colorimetrically with potassium thiocyanate, this method being preferable to titration with potassium permanganate for the small amounts present.

ANALYTICAL RESULTS

In Table I are given the data on the weight and composition of a single plant with respect to withered leaves, etc., at each period of sampling. The weights of the plants were, of course, accurately determined, the probable error of the weights and percentages of dry matter merely show-

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

ing the degree of accuracy with which each sample represented, in respect to weight and moisture content, the average of all the plants at each period. In calculating the probable error, one cylinder of 29 plants was taken as a unit. The development of the plants at the different stages was as follows: At 18 days the plants were stooling to some extent; at 73 days they were just about to flower; at 103 days panicles were out, but the seeds were only partially formed; at 123 days seeds were fully formed and ripe.

TABLE I.—*Weights of the different parts of the upland rice plant analyzed at various periods*

Age of plant.	Dry weight of green stalks and leaves per plant.	Dry weight of withered stalks and leaves per plant.	Dry weight of panicles per plant.	Dry weight of whole plant above ground per plant.	Percentage of dry matter in whole plant aboveground.
Days.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
18	0.132	.....	.....	0.132	18.7
26	.581	.....	.....	.581±0.035	20.2±0.08
48	4.38	.....	.....	4.38 ±0.25	14.6±0.05
73	11.47	0.95	.....	12.42 ±1.20	20.9±0.10
103	21.76	4.53	3.24	29.53 ±0.99	25.8±0.99
123	.....	23.34	12.12	35.46 ±1.90	36.2±0.16

It will be noted that the percentage of dry matter in the green plant did not rise until the plant had begun to form seeds. Previous to this time the percentage of dry matter in the plant was somewhat irregular, but tended to remain about 20 per cent. The variations in the moisture content of the first four samples are so many times the probable error of each result that they could not be due to poor sampling. There is little doubt that weather conditions affected the amount of moisture or dry matter in the green plant during the first four stages of growth, while the moisture content of the last two samples was controlled chiefly by the physiological changes in the plant—accumulation of carbohydrates and death of old leaves. This seems evident from the records of rainfall. During the eight days preceding the cutting of each sample the number of days with rain and the total precipitation for the eight days were as follows: Previous to the 18-day-old sample, 5 days with rain, 2.80 inches; previous to the 26-day-old sample, 1 day with 0.90 inch; previous to the 48-day-old sample, 6 days with 4.63 inches; and previous to the 73-day-old sample, 1 day of rain with a precipitation of 0.32 inch. The weather was thus relatively wet, dry, wet, dry; and the percentages of moisture in the green plant were respectively high, low, high, low.

The ash analyses of the various samples are given in Table II. The panicles included the seeds and supporting stems. Withered leaves of the 73- and 103-day-old samples were analyzed separately from the green leaves and stalks, but no such separation was made for the 123-day-

old sample, as all the leaves and straw were partially or completely withered at this period. The 18- to 48-day-old samples had no withered leaves, so that these analyses represent the whole plant aboveground.

TABLE II.—*Ash analyses of vegetative parts of the rice plant at various periods*

Part of plant.	Age of plant.	Percentage of dry matter in green sample.	Percentages in dry matter of—		Percentages in carbon-free ash of—								
			Carbon-free ash.	Nitrogen (N).	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl).
Green leaves and stalks	*Days.* 18	18.7	17.75	.....	56.88	2.21	3.69	0.75	22.91	1.55	7.94	9.24	.....
Do	26	20.2	14.97	4.02	56.34	2.49	4.24	.35	17.28	9.76	6.65	5.81	4.47
Do	48	14.6	22.21	1.89	62.56	1.73	3.11	.21	14.66	9.57	4.00	4.47	4.10
Do	73	20.1	16.95	1.93	64.82	1.73	3.04	.18	16.88	4.55	3.39	3.89	5.37
Do	103	21.5	13.28	1.01	67.71	2.12	3.26	.11	9.54	10.31	4.96	3.61	4.52
Withered leaves and stalks	123	28.7	20.23	.62	74.00	1.83	2.27	.32	12.65	4.85	1.80	2.52	3.26
Withered leaves	73	37.4	30.12	1.16	83.51	3.12	3.08	.97	6.44	1.31	1.33	1.73	1.69
Do	103	61.0	27.60	.41	85.10	3.00	2.84	.72	2.74	2.33	.67	1.20	.80
Panicles immature	103	48.5	8.86	1.18	78.15	1.17	2.68	.07	6.80	3.20	6.91	3.72	.68
Panicles with ripe seed	123	72.8	4.82	1.26	68.93	1.31	4.91	.06	9.74	1.04	14.67	5.60	.....

The percentage of iron in the ash of the green straw and leaves decreased regularly and rapidly with the maturity of the plant, the greatest decrease being from the 18-day-old to the 26-day-old sample.[1] The withered leaves had a relatively high percentage of iron. This may be due to the other samples, consisting of both leaves and stalks, or to the fact that the withered leaves of the 73- and 103-day-old sample were the leaves that appeared first—i. e., those forming a large part of the first samples.

The varying percentages of iron in the ash of the green straw and withered leaves agree with some of the results obtained by Arendt[2] with oats. He found that the lower leaves of wheat, which must have been withered at the later periods of analysis, contained increasing percentages of iron, which were much greater than the percentages of iron in the ash of the upper leaves.

The lower percentages of potash, phosphoric acid, sulphur, chlorin, and nitrogen in the ash of the withered leaves may be due to translocation of these elements preceding death of the leaves or to loss by leaching after death of the tissue.

In Table III is given the ash composition of the roots and of the whole plant aboveground. The roots for analysis were washed with great care,

[1] These results are in accord with many analyses of green rice straw made previously. Four samples of rice straw from plants grown in four different soils for 25 days contained from 2.76 to 1.98 per cent of iron (Fe_2O_3) in the ash, while samples from a crop grown 84 days had 0.31 to 0.18 per cent, and samples from a 129-day-old crop had but 0.12 to 0.10 per cent of Fe_2O_3 in the ash. (Gile, P. L., and Ageton, C. N. The effect of strongly calcareous soils on the growth and ash composition of certain plants. Porto Rico Agr. Exp. Sta. Bul. 16, p. 31, 1914.)

[2] Arendt, R. F., Untersuchungen über einige Vorgänge bei der Vegetation der Haferpflanze. *In* Landw. Vers. Stat., Bd. 1, p. 31–36. 1859.

but it was impossible to wash them white. The analyses show that the material which could not be washed off was probably finely divided ferric oxid. The percentages of iron found in the ash of the roots ranged from 5.36 to 8.48. This was obviously due to iron contamination from the soil. It was evident, however, that this was a selective contamination chiefly of iron particles, as the ratio of Fe_2O_3 to Al_2O_3 to SiO_2 in the soil was about 1 to 1.5 to 6.[1] Thus, a contamination of the soil as such which would have increased the iron content 6 per cent would have raised the silica 36 per cent and the alumina content 9 per cent. As the high iron content of the root ash is thought to be due to selective contamination from the soil, the results for iron are not reported. The percentages of the other constituents, except possibly silica, could not have been materially affected by soil contamination.

TABLE III.—*Ash composition of the roots and of the whole rice plant aboveground*

Material analyzed.	Age of material.	Percentages in carbon-free ash of—								
		Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl_2).
	Days.									
Whole plant aboveground.	18	56.88	2.21	3.69	0.75	22.91	1.75	7.94	9.24	.....
Do	26	56.34	2.40	4.24	.35	17.28	9.76	6.65	5.81	4.47
Do	48	62.56	1.73	3.11	.21	14.66	9.57	4.00	4.41	4.10
Do	73	67.24	1.89	3.07	.28	15.54	4.13	3.10	3.61	4.90
Do	103	73.29	2.30	3.09	.28	7.46	7.60	3.87	2.94	3.22
Do	123	73.43	1.77	2.57	.29	12.33	4.43	3.21	2.86	2.90
Roots	18	42.28	3.82	9.68	.....	22.53	2.10	7.33	.....	.....
Do	26	35.62	3.75	8.42	.....	15.46	17.23	8.11	5.48	.....
Do	48	46.06	3.01	4.36	.....	21.03	6.32	4.98	8.06	2.30
Do	73	60.21	2.84	4.30	.....	15.24	3.74	3.02	6.73	1.92
Do	103	61.57	2.76	3.84	.....	10.83	4.42	2.46	6.67	.99
Do	123	64.70	4.31	3.05	.....	12.47	1.19	2.63	6.87	1.45

The percentages of iron in the ash of the whole plant aboveground showed but little variation after the sharp drop from the 18- to the 26-day-old sample.

Leaving out of consideration the 123-day-old sample, the composition of which was probably influenced appreciably by the leaching of rain, it can be seen that during the growth of the plant the percentages of lime and magnesia in the ash tended to remain constant, the silica increased, the phosphoric acid and sulphuric acid decreased, the potash, somewhat irregular, tended to decrease, and the soda was irregular. The variations in the percentages of soda are somewhat peculiar, the increase from the 18- to 26-day-old sample being out of all proportion to changes in other constituents. Soda in the ash of the roots, however, increased to an

[1] Iron is much higher in the finer soil separates than in the coarser. (Failyer, G. H., Smith, J. G., and Wade, H. R. The mineral composition of soil particles. U. S. Dept. Agr. Bur. Soils Bul. 54, 36 p. 1908.)

equally great extent from the 18- to 26-day-old sample. Variations in the percentages of potash in the ash of the plant aboveground were for the most part accompanied by similar variations in the ash of the roots. The percentages of soda in the ash seem, as a rule, to fluctuate inversely as the percentages of potash. This is in accord with results showing that soda can to a small extent replace or exercise part of the functions of potash.[1]

In the ash of the roots lime, magnesia, phosphoric acid, and chlorin all decreased fairly regularly with the age of the sample.

In Table IV are given the percentages of the ash constituents present in the dry matter of the roots and of the whole plant aboveground.

TABLE IV.—*Ash constituents in dry substance of the roots and the whole rice plant aboveground*

Material analyzed.	Age of material.	Percentage of dry matter in whole plant aboveground.	Percentages of ash constituents in dry substance of plant.										
			Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin (Cl).	Nitrogen (N).
Whole plant aboveground	*Days.* 18	18.7	17.75	10.10	0.39	0.65	0.133	4.07	0.28	1.41	1.64	.....	.....
Do	26	20.2	14.97	8.43	.36	.63	.052	2.59	1.47	1.09	.87	0.67	4.02
Do	48	14.6	22.21	13.89	.38	.69	.048	3.26	2.13	.89	.98	.91	1.89
Do	73	20.9	17.96	12.07	.34	.55	.051	2.79	.74	.56	.65	.88	1.87
Do	103	25.8	14.99	10.99	.35	.46	.041	1.12	1.14	.58	.44	.48	.94
Do	123	36.2	14.96	10.99	.26	.38	.044	1.85	.66	.48	.43	.43	.84
Roots	18	.....	11.71	4.95	.45	1.13	.....	2.64	.25	.86	.....	.....	.....
Do	26	.....	9.49	3.38	.36	.80	.....	1.47	1.64	.77	.52	.....	1.48
Do	48	.....	7.82	3.60	.24	.34	.....	1.64	.49	.39	.63	.18	.95
Do	73	.....	8.32	5.01	.24	.36	.....	1.27	.31	.25	.56	.16	1.09
Do	103	.....	8.09	4.98	.22	.31	.....	.88	.36	.20	.54	.08	.73
Do	123	.....	5.53	3.58	.24	.17	.....	.69	.07	.15	.38	.08	.66

In the first four samples the percentages of ash in the dry matter of the plant aboveground varied inversely as the percentages of dry matter in the green plant, and, as noted above, the percentages of dry matter seemed to be lower during the periods of greater precipitation. Thus, with dry weather preceding the sample, the percentage of dry matter in the green plant was high and the percentage of ash low.[2] An average of several crops of rice grown at different times to eliminate the effect of temporary weather conditions would doubtless show gradually increasing percentages of dry matter in the green plant and gradually decreasing percentages of total ash in the dry matter.

[1] Wagner, Paul. Forschungen auf dem Gebiete der Pflanzenernährung. I. Theil: Die Stickstoffdüngung der Landwirthschaftlichen Kulturpflanzen. p. 232, Berlin, 1892.

Hartwell, B. L., and Pember, F. R. Sodium as a partial substitute for potassium. *In* R. I. Agr. Exp. Sta. 21st Ann. Rpt., 1907–1908, p. 243–247. 1908.

[2] This is probably owing to the fact that during wet weather the growth of new leaves and tissues is especially active, while in dry weather organic matter is formed more rapidly than mineral matter is absorbed.

On account of the fluctuations in the amount of total ash, it is thought that the percentages of the various ash constituents in the dry matter are less significant than the composition of the ash, which would be unaffected by temporary weather conditions.

The plants were not analyzed at frequent intervals while ripening; nevertheless, the preceding work throws some light on the question of loss of mineral elements at this time. In Table V are given the absolute weights of the ash constituents in one plant at 103 and at 123 days.

TABLE V.—*Gain or loss of ash constituents by the rice plant aboveground during last 20 days of growth*

Material analyzed.	Age of material.	Weight of ash constituents (in grams) in one whole plant aboveground.									
		Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Iron (Fe_2O_3).	Potash (K_2O).	Soda (Na_2O).	Phosphoric acid (P_2O_5).	Sulphuric acid (SO_3).	Chlorin Cl).	Nitrogen (N).
	Days.											
Whole plant aboveground..	103	4.427	3.245	0.102	0.137	0.012	0.330	0.337	0.172	0.277	0.130	0.143
Do.....	123	5.306	3.896	.094	.137	.015	.655	.235	.170	.297	.152	.154

It is evident that the aboveground part of the plant lost considerable soda between the last two periods. The roots also must have lost considerable soda, as the percentage of soda in the dry matter of the roots dropped from 0.36 per cent at 103 days to 0.07 per cent at 123 days, while the absolute weight of roots could have increased but little during this interval. The results do not show whether there was any loss of the remaining ash constituents. It is only apparent that, as compared with 103 days, the plant aboveground contained at 123 days the same or a slightly greater quantity of all ash constituents except soda. It is, of course, possible that between 103 and 123 days there might have been an increase followed by a loss of the other ash constituents. The marked loss of soda was more than compensated for by a gain in potash. The increases in the other elements were relatively slight, and the apparent losses of lime and phosphoric acid are without significance when the probable errors of the weights of the plant at the two periods are considered.

DISCUSSION OF RESULTS

It is unnecessary to detail all the changes in ash composition that occurred during the growth of the plant, as these are evident in the tables. In common with similar studies of many other plants the percentages of potash, phosphoric acid, and sulphur in the ash and of nitrogen in the dry matter decreased with the age of the plant, while the silica increased.

The results show that while the iron content of the ash of the whole plant varied but little with the age of the plant, the percentage of iron in

the ash of the green straw and leaves decreased markedly with its age. The withered leaves and straw thus contain a much greater percentage of iron in the ash than the active or live parts of the plants. This would indicate that iron, like silica, is not transported or leached from the dead tissue to the same extent as the other mineral elements.

SUMMARY

Ash analyses of upland rice were made at intervals to show the ash composition of the plant, especially in regard to iron content, from an early stage to complete maturity.

The percentages of potash, phosphoric acid, and sulphur in the ash of the whole plant aboveground decreased with the age of the plant, while silica increased and nitrogen in the dry matter decreased with the age.

As compared with 103 days, when the panicles were just out, the mature plant aboveground at 123 days with the seeds ripe contained an equal amount of lime, magnesia, and phosphoric acid, slightly more iron, sulphur, chlorin, nitrogen, and silica, much less soda, and considerably more potash.

The percentages of iron in the ash of the green leaves and straw decreased regularly and markedly with the age of the plant, while the percentages of iron in the ash of the whole plant aboveground remained fairly constant after the 26-day-old sample.

Previous to flowering, the percentages of dry matter in the green plant and of ash in the dry matter seemed to be influenced by the effect of the weather on the growth of the plant.

VARIETAL RESISTANCE OF PLUMS TO BROWN-ROT

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INTRODUCTION

In the control of plant parasites a great deal of attention has recently been paid to the possibilities of producing resistant plants by breeding. In the plum-breeding plots of the Minnesota Fruit-Breeding Station at Excelsior it is very noticeable that the fruit of certain seedling varieties of plums (*Prunus* spp.) appears to rot much more readily than that of others. The rot is due to attacks of the brown-rot fungus, *Sclerotinia cinerea* (Bon.) Wor. As a knowledge of the factors controlling resistance is necessary for intelligent effort in breeding work, a study of the resistance of plums to the brown-rot fungus was begun in the spring of 1913. The following is a report of the results obtained on the nature of parasitism of the fungus and on varietal resistance of plums to the fungus.

HISTORICAL SUMMARY

TAXONOMIC REVIEW

The life history of the brown-rot fungus has been rather completely worked out, both in this country and in Europe. Woronin (1900)[2] made a very complete comparative study of *Monilia fructigena* and *M. cinerea*. Two years later Norton (1902) discovered and described the apothecial stage of the American form and referred *M. fructigena* Persoon to *S. fructigena* (Pers.) Schröter. Shortly after this, Aderhold and Ruhland (1905) found and described a perfect stage of *Sclerotinia* spp. on apples, which they concluded to be that of *M. fructigena*. They also found a perfect stage of the apricot brown-rot fungus, *M. laxa*, the Monilia stage of which can not be distinguished morphologically from that of *M. cinerea*. A comparison of the perfect stage of the apricot fungus with the perfect stage of the peach fungus of this country, sent to them by Norton, showed differences in ascus and ascospore sizes, and these, with the slight differences which they found in the ability of the two species, *S. cinerea* and *S. laxa*, to infect plum flowers, led them to the conclusion

[1] The work was carried on under direction of the Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota. The writer wishes to acknowledge indebtedness for suggestions, assistance, and criticism to the following: Dr. E. M. Freeman and Dr. E. C. Stakman, Prof. R. W. Thatcher, of the Division of Chemistry, and Dr. M. J. Dorsey, of the Division of Horticulture, in whose laboratory the work was carried on. The writer also wishes to express his appreciation of the assistance rendered by Mr. Ernest Dorsey in the photomicrographic work and to Dr. C. O. Rosendahl for suggestions and the use of apparatus.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 392-395.

that the fungus found on the apricot was a species (*S. laxa*) distinct from that found on plums and cherries (*S. cinerea*). They also concluded that the American species must be *S. cinerea*. A comparison of the ascospores of *S. cinerea* with those of *S. fructigena* brought out the fact that the former always contain from one to many oil globules, while the latter contain none.

Pollock (1909), in a study of the Michigan brown-rot fungus, concluded that it was probably the same species which Norton described, and that, so far as the chlamydospore measurements were concerned, it resembled *S. cinerea* more than *S. fructigena*. Pollock also showed that the microconidia observed by Woronin (1888) on certain other species of Sclerotinia and by Humphrey (1891) as appearing on plums which did not produce spore tufts were also produced in abundance when ascospores of the American brown-rot fungus were germinated in distilled water.[1]

An important taxonomic fact was brought out by Ewert (1912) when he showed that the Monilia spores of *S. fructigena* would not live over the winter, while those of *S. cinerea* would. This difference was not due to the effects of cold, as the spores of *S. fructigena* would stand low temperatures. That the spores of the American form would live over the winter was shown by Arthur (1886), who on May 8 germinated spores taken from mummies of cherries which had hung on the tree all winter. Galloway (1889), in May, 1888, germinated spores taken from mummies collected in July, 1886.[2]

The perfect stage of the cherry brown-rot fungus in Europe was not found until 1912. Westerdijk (1912) described it at this time and concluded (p. 41), from ascus and ascospore measurements, that "Neben den 3 beschriebenen Obstsclerotinien ist dann also eine spezielle Kirschensclerotinie aufzustellen." The asci and ascospore measurements presented by Reade (1908) and Pollock (1909), however, do not warrant this conclusion.

Matheny (1913) made an extensive study of the brown-rot fungus from various parts of this country and compared it closely with pure cultures of *S. fructigena* and *S. cinerea* sent to him from Europe. He concluded that the Monilia stage in this country agreed very closely with that of *S. cinerea* of Europe and that the apothecial stage differed in shape of spore and in the presence of oil globules in the ascospores from that of *S. fructigena* and referred the American brown-rot fungus to *S. cinerea*. Conel (1914) made a study of the brown-rot in the vicinity of Champaign and Urbana, Ill., and decided, both because of its morphological characters and from the fact that the Monilia form is capable of living over winter, that the fungus was *S. cinerea*.

[1] Jehle in an unpublished thesis on file at the University of Minnesota also observed the production of these conidia from ascospores, and on the same hypha observed the Monilia spores, thereby definitely connecting the perfect and the Monilia stages.

[2] Jehle also germinated spores found on mummies in the early spring.

PHYSIOLOGICAL REVIEW

A considerable amount of literature has appeared, especially in recent years, on the subject of resistance and immunity to disease. The cereal crops have perhaps received the most attention. Bolley (1889) and Anderson (1890) attempted to correlate resistance with certain morphological characters. Cobb (1892, p. 181–212) advanced the theory of mechanical resistance due to morphological characters, such as thick cuticle, waxy coating, and small stomata. Freeman (1911) showed that barley might escape rust owing to variation in amount of bloom produced on the leaves, which could be varied by growing in soils of different degrees of alkalinity. This escape from rust is not true resistance, but is due to the inability of the water to wet the surface of the leaves so that the drops containing the spores roll off. When these plants were infected, however, they "exhibited large and vigorous growths of the rust."

Marryat (1907) showed in the case of *Puccinia glumarum* grown on a semi-immune host that it killed small areas of the host tissue and formed only small or abortive pustules, while in the case of the susceptible forms the host cells, though containing haustoria, were apparently normal.

Comes (1912) reported that Rieti wheat, which is very resistant to rust, contained a higher percentage of acid than other more susceptible forms and also that the acid content increases with the altitude at which wheats are grown, as does also the ability to resist rust.

Jones (1905) showed that some varieties of potatoes are much more resistant to certain potato diseases than others. He based resistance more on chemical composition than on morphological differences in the host.

Kinney (1897) noted that "fruit of different varieties of plums varies in susceptibility to injury by rot fungus" and attributed the difference in resistance to variations in texture of the skin. He also stated that early varieties are usually injured more than those which ripen their fruit later.

Müller-Thurgau (1900) noticed that varieties of apples in Switzerland showed different degrees of susceptibility to a wilt or blight caused by *M. fructigena.*

Quaintance (1900) observed a marked variation among varieties of drupaceous fruits in their resistance to attacks of the brown-rot fungus. Among the peaches the varieties densely covered with down were the most susceptible. Of the plums some varieties of the Miner group were practically free, those of the Wild Goose rotted about 10 per cent, while the varieties of *Prunus americana*, *P. triflora*, and *P. pumila* were very susceptible. He suggested that the firmness and thickness of the skin of the Miner plums might have something to do with their resistance. The relative resistance of some varieties of *P. domestica* to brown-rot is given by Alwood and Price (1903).

Köck (1910) ascribes the resistance of certain varieties of cherries to a blossom-blight caused by *S. cinerea* to the blossoming of these varieties when conditions are unfavorable for the disease.

Cook and Taubenhaus (1911 and 1912) pointed out the toxic properties of tannins and fruit acids and also showed a relationship to exist between the decrease in oxidizing enzym content of fruits and the increase in their susceptibility to disease.

With regard to the physiological relationship between host and parasite, considerable work has been done. Jones (1910) gave a comprehensive review of the literature on this subject, dealing especially with the bacteria. Cooley (1914) reviewed much of the work on the physiological relations of the fungi. Therefore, only a short review will be given of the literature dealing with *Sclerotinia* spp.

Behrens (1898) in his work on the physiology of *Oidium* (*Sclerotinia*) *fructigenum*, *Penicillium* spp., and some other fungi, concluded that *S. fructigenum* was exclusively an intercellular fungus and did not secrete a cellulose-dissolving enzym. He considered that the fungus split the middle lamella by mechanical force. *Penicillium* spp., he concluded, also did not enter the cells, but did produce a middle-lamella-splitting enzym.

Schellenberg (1908) studied the effect of *S. fructigena* and *S. cinerea* on a number of tissues, but not on their respective hosts. He considered both of these fungi to be intercellular, producing no cellulose-splitting enzym. He thought, however, that they did produce a hemicellulose-dissolving enzym and that the cell walls in contact with the hyphæ were slightly dissolved. He saw no evidences of a middle-lamella-splitting enzym.

Bruschi (1912) noticed, when *M. cinerea* was grown in a medium containing plum flesh, that after 48 hours the cells were all separated from one another, and concluded that the fungus produced the middle-lamella-splitting enzym pectinase. Attempts to isolate a cellulose-dissolving enzym were unsuccessful.

Cooley (1914) demonstrated the ability of *S. cinerea* to produce an enzym which would coagulate pectin from solution in the absence of calcium. This enzym he called "pectinase." His use of this term is, however, not clear, as he states (p. 314) that he adopted "the nomenclature used by Jones and Euler, namely, employing pectinase as the term to designate the enzyme inducing coagulation of a pectin solution and also the hydrolysis of calcium pectate, or pectinate." Jones (1910) used, in a general way, the nomenclature suggested by Bourquelot and Hérissey (1898) regarding the enzym which they extracted from barley malt; as he says (p. 355), "All things considered, we favor the name *pectinase*, which was suggested by Bourquelot and Hérissey, as already explained." On the other hand, Euler-Chelpin (1912, p. 32) states that "The enzyme

here termed pectase was obtained from malt-extract by Bourquelot and Hérissey, who called it pectinase; according to the general principle of naming enzymes after the substrate, this should be altered to pectase." In a subsequent paragraph he states that "By the term pectinase should be indicated the enzyme which coagulates dissolved pectin substances, e. g., in fruit juices, in the presence of lime to gelatinous calcium salts of the feebly acid pectinic acids." If we follow the definition of a pectinase given by Jones and the classification given by Haas and Hill (1913, p. 339), we must refer to the enzym demonstrated by Cooley as "pectase."

The attempts of Cooley (1914) to isolate a middle-lamella-splitting enzym from rotted fruit gave negative results. In certain artificial media a cellulose-dissolving enzym was produced, but its action on cellulose isolated from plums was very slight. From direct observations on the fungus in free-hand sections of fruit he concluded that "the fungus does not show any particular affinity for the middle-lamella, but penetrates and permeates with equal avidity any part of the host tissue." He could find no relationship to exist between varying acid content of plums at different periods of development and increased susceptibility of ripe over green fruits.

EXPERIMENTAL MATERIAL

The organism used in this work was isolated when needed from rotting plums, as it seemed better to use only strains which had been growing under normal conditions rather than to risk a decrease in virulence of infection due to growing a single strain on artificial media.

The plums used consisted for the most part of hybrids produced at the Minnesota Fruit-Breeding Station at Excelsior. Those referred to in the text as "B × W" are hybrids of Burbank (*P. triflora*), the female parent, with Wolf (*P. americana mollis*). The A × W crosses are Abundance (*P. triflora*) × Wolf. The Burbank is a medium thick-skinned variety which becomes soft when ripe and is rather susceptible to the brown-rot. Wolf has a thick, tough skin and is not affected to any great extent by the rot in the field. Abundance is reported by Hedrick (1911) as being less subject to attacks of the brown-rot than Burbank. The crosses B × W15 and A × W18 are both characterized by being very firm when ripe, and are both nearly immune to brown-rot in the field. The other hybrids of these two series vary in firmness and resistance.

Etopa and Sapa (*Prunus besseyi* × Sultan, *P. triflora*) and Wakapa (Red June, *P. triflora*, × DeSota, *P. americana*, but resembling very closely a sand-cherry hybrid) are products of the South Dakota Experiment Station. They are thin-skinned varieties and are susceptible to rot. The sand cherry (*P. besseyi*) is a small fruit which becomes soft on ripening. It has very astringent flesh and is susceptible to brown-rot.

Gold is a thin-skinned susceptible variety. Sultan is not known to the writer.

The three varieties designated "S. D. Nos. 1, 2, and 3" are varieties obtained from Mr. A. Brackett, of Excelsior, who received them from the South Dakota Experiment Station. Their true names were not known to Mr. Brackett. S. D. No. 1 is a thin-skinned variety and rotted badly on the trees when sprayed once with Bordeaux mixture. S. D. Nos. 2 and 3 were thicker skinned, firmer varieties and did not rot after one spraying, many fruits drying on the trees. All appear to be sand-cherry hybrids.

Compass, a hybrid between a sand cherry and *P. americana* (Hansen, 1911), is a thin-skinned variety which becomes soft on ripening and is susceptible to the brown-rot. Reagan, a hybrid of Wayland (*P. hortulana*) × *P. americana* (Hedrick, 1911) is thick-skinned, very firm when ripe, and is very resistant to the rot. Specimens of the ripe fruit used were received from the New York Experiment Station, Geneva, N. Y.

Ocheeda and Harrison are varieties of *P. americana*. Manitoba No. 1 is probably a variety of *P. nigra*. Hammer is a hybrid between *P. hortulana mineri* and *P. americana* (Hedrick, 1911). These varieties were obtained from the orchard at University Farm.

TAXONOMY OF THE FUNGUS

MONILIA STAGE

The brown-rot fungus in Minnesota is found for the most part affecting plums, but to a very limited extent also attacking the apple. It appears on the plum first as a small brown or purple spot, which increases very rapidly in size. In a very short time the spore tufts appear irregularly over the surface of the rotted area. These are usually small and ashen gray in color, although in many cases the color varies to a yellow ocher. Plums inoculated through a wound made by cutting off the tip of the fruit, when allowed to rot under a cardboard box in nearly total darkness, produced spores of a bright-ocher color over the wounded area and in some cases through the skin. Mummies collected from trees in the late fall showed spore tufts which varied from gray to a light ocher. The chlamydospores of the local form, taken from mummies which have hung on the trees over winter, retain their power of germination.

Chlamydospore measurements were made of spores from Soulard and Longfield apples, from Harrison, Ocheeda, Newman, and Surprise plums, which were rotted in the laboratory, and from a culture on beerwort agar. In each instance 100 spores were measured, except in the case of the beerwort-agar culture, where 50 spores were measured. The results are given in Table I.

TABLE I.—*Chlamydospore measurements of Sclerotinia cinerea*

Medium.	Average length.	Average breadth.	Medium.	Average length.	Average breadth.
	μ	μ		μ	μ
Surprise plum	16.22	11.24	Longfield apple	15.80	10.81
Newman plum	17.38	12.10	Soulard apple	15.30	10.76
Ocheeda plum	16.18	11.09	Beerwort agar	14.05	8.77
Harrison plum	15.95	10.98			

From a comparison of these measurements with those given in Table II, it will be seen that they agree very closely with those obtained by other investigators in this country and are only slightly larger than those given for *S. cinerea* by European investigators. They also correspond closely to the measurements given by Aderhold and Ruhland for *S. laxa* found on apricots.

TABLE II.—*Spore and ascus measurements of the brown-rot fungus as given by various investigators*

FROM EUROPEAN SOURCES

Fungus and investigator.	Host.	Chlamydospores.	Asci.	Ascospores.
Sclerotinia cinerea:		μ	μ	μ
Saccardo (1886)	.....	15 to 17 by 10 to 12	.....	.....
Woronin (1900)	.....	12.1 by 8.8 to 13.2 by 9.9	.....	.....
	In culture	17.5 to 24.2 by 11.2 to 13.2	.....	.....
Aderhold and Ruhland (1905)	Cherry	13.8 by 9.2	.....	.....
	Various	13.8 by 9.95	.....	.....
Matheny (1913)	Peach and plum	14.4 by 10.8	.....	.....
Sclerotinia laxa:				
Aderhold and Ruhland (1905)	Apricot	16.1 by 10.8	121.5 to 149.9 by 8.5 to 11.8	11.5 to 13.5 by 5.2 to 6.9
Cherry brown-rot:				
Westerdijk (1912)	Cherry	.....	158.4 to 171.6 by 7.9 to 8.5	13.2 to 16.8 by 4.3 to 5.2
Sclerotinia fructigena:				
Saccardo (1886)	.....	25 by 10 to 12	.....	.....
Woronin (1900)	Apple	20.9 by 12.4 to 24.5 by 13.2	.....	.....
	In culture	23.7 to 30.8 by 14.9 to 16.5	.....	.....
Aderhold and Ruhland (1905)	Apple	25 by 13	120 to 180 by 9 to 12	11 to 12.5 by 5.6 to 6.8
Matheny (1913)	.....	22.1 by 11.2	.....	.....

TABLE II.—*Spore and ascus measurements of the brown-rot fungus as given by various investigators*—Continued

FROM AMERICAN SOURCES

Fungus and investigator.	Host.	Chlamydospores.	Asci.	Ascospores.
			μ	μ
Sclerotinia fructigena: Norton (1902)			45 to 60 by 3 to 4	
Aderhold and Ruhland (1905)			89.3 to 107.6 by 5.9 to 6.8	6.2 to 9.3 by 3.1 to 4.6
Reade (1908)		17 by 11	125 to 215 by 7 to 10	10 to 15 by 5 to 8
Pollock (1909)	Plum	14.4 to 24 by 9.6 to 14.4	130 to 179 by 9.2 to 11.5	11.4 to 14.4 by 5 to 7
	In culture	9.6 to 14.4 by 7.2 to 10.8		
Matheny (1913)		14.7 by 9.9		
	Peach		135 to 190 by 6.9 to 10.5	10.5 to 14.5 by 5.2 to 7.5
	Plum		135 to 173 by 6.8 to 10.8	9.3 to 14.2 by 5 to 7.4

SCLEROTINIA STAGE

The apothecial stage of the local brown-rot fungus has been found in abundance in the University of Minnesota Experiment Station orchard during the last few springs. It appears during the blooming period of the plums. The ascospores showed the characteristic refractive globules which Aderhold and Ruhland (1905) pointed out as being one of the characters which make it possible to distinguish between *S. cinerea* and *S. fructigena*, the latter species containing none.

Some doubt has existed in regard to the exact time required for the production of the perfect stage after the formation of the sclerotium or mummy. The field observations of Norton (1902) and others seem to indicate that the apothecia are formed the second spring after the rotting of the fruit—i. e., in approximately 18 months. Other investigators (Dandeno, 1908) have thought that they may be produced the spring following the rotting of the fruit. No experimental evidence has come to the notice of the writer which shows definitely the period required for the production of apothecia; therefore, the following experiment was performed.

During the fall of 1913 two lots of mummied plums and one of apples were buried. Lot 1 consisted of 1 plum each of 16 varieties which had been rotted in the laboratory. These were buried on October 8, 1913, about ½ to 1 inch deep in a shallow box, which was then placed level with the ground on a shaded hillside. Lot 2 consisted of (A) 106 fruits from 8 varieties of plums which had rotted in the field under field conditions during the fall of 1913, and (B) 30 mummies of 3 other varieties which

had been hanging on the trees since the fall of 1912. The plums of this lot were buried on October 15, 1913, near the previous lot and when finally examined were buried from ¼ to 1 inch deep. The fruit of each variety was kept separate. Lot 3 was made up of 48 apples representing 7 varieties. The fruits had been inoculated through wounds in the laboratory and on October 18, 1913, when entirely rotted, were buried.

The results obtained were as follows: In the spring of 1914 no apothecia were found on any of the three lots. An examination of lot 1 on May 7, 1915, showed 4 of the total of 16 fruits producing a total of 71 cups. On further examination these were all found to be growing from the upper side of the sclerotium. Two others, which had been buried deeper, were found to be producing many of the young cups which at this time had not reached the surface of the ground.

Lot 3 at this time showed no apothecia. On May 12, 1915, lot 2 was examined; of the total of 106 mummies produced in 1913, 39 were producing apothecia in abundance. In a number of other instances the sclerotium was present, but was producing no apothecia. Of the 30 mummies produced in 1912, 4, of the Opata variety, were producing a total of 10 cups, while the sclerotia of the Compass and Topa varieties had entirely rotted. At this time lot 3 was also examined, and as no apothecia were being produced an attempt was made to find the sclerotia. Small pieces of the black, leather-like sclerotia were found where 4 of the varieties had been buried, but in all other cases they had entirely rotted. The sclerotium of a Shields crab-apple had a growth of about one-fourth of an inch upon it which appeared very much like that of a young cup, but when this piece was again buried it showed no further development.

From this experiment we may conclude that for the production of the perfect stage of *S. cinerea* the mummies must be buried for at least two winters and that mummies which have hung on the tree for one year still have the power of producing apothecia.

From a horticultural standpoint it is of interest to note that of the 156 plum pits buried in 1913 none germinated in the spring of 1914, but in the following spring 106 produced young plants. Of these, 6 were of the Topa variety which had hung on the tree for one year before burying.

Measurements were made of asci and ascospores from material collected on April 10, 1914. The asci varied in length from 102 to 166μ, and in breadth from 3.5 to 5.7μ. The ascospores varied from 5.6 to 8.9μ in length and from 2.9 to 3.8μ in breadth.

Reference to Table II shows the wide range in ascus and ascospore measurements as determined by various investigators, the asci of Norton ranging from 45 to 60 by 3 to 4μ; of Aderhold and Ruhland (who received their material from Norton), 89.3 to 107.6 by 5.9 to 6.8μ, those from the Minnesota Expetiment Station, 102 to 166 by 3.5 to 5.7μ, while the upper extreme is reached by Reade (who also obtained his material from Norton), who found the asci ranging from 125 to 215 by 7 to 10μ.

By comparing the figures given by Westerdijk (1912) for the cherry fungus with those given above, it will be seen that they fall well within the range of *S. cinerea*, and as this difference in the size of the asci and of the ascospores was the only one upon which she based her conclusion as to its being a separate form, it seems safe to conclude that what she described was the perfect stage of *S. cinerea*.

It has already been pointed out that the Monilia stage of the apricot fungus, described by Aderhold and Ruhland (1905), compares favorably with the Monilia stage of the American brown-rot fungus, and they showed that it was identical, except for slight differences in chlamydospore size, with that of the European *S. cinerea*. By referring to Table II it will be seen that the ascus and ascospore measurements given for the perfect stage of *S. laxa* fall well within the limits determined for *S. cinerea*. Considering the fact that at present there are no known morphological differences between *S. cinerea* and the apricot fungus, is the fact that Aderhold and Ruhland were able to get infection of plum flowers in only a few cases with chlamydospores of *S. laxa* sufficient evidence to make this a separate species?

MICROCONIDIAL STAGE

The microconidial stage, as was stated above, has been described by Woronin for a number of species of Sclerotinia, including *S. fructigena* and *S. cinerea*. He, however, could show no differences between the spores of the two latter species, and they are therefore of little value in identification of the species.

The production of the microcondia was first seen by the writer in a potato-plug culture of the local fungus nearly a year old. The spores ranged from 2.2 to 2.6μ in diameter, were spherical, and contained a large refractive globule. They were later found on agar cultures in great abundance, in hanging drops of distilled water, and also in hanging drops of 1 per cent malic, 0.062 gallic, 0.062 and 0.25 per cent tannic acids. In the latter cases the flask-shaped sterigmata could be seen. Chains of from 15 to 20 spores were not uncommon. They were also produced in great abundance on the surface of a very young Surprise plum picked and inoculated June 3. These spores ranged in size from 2.55μ to 3.22μ, averaging for 25 measurements 2.72μ. The microconidia produced in the 1 per cent malic-acid solution were larger, ranging from 2.60 to 3.79μ, measurements of 25 spores averaging 3.14μ.

PHYSIOLOGICAL AND PATHOLOGICAL RELATIONS

INFECTION

Opinions differ as to the ability of the brown-rot fungus to penetrate the uninjured surface of fruits. Peck (1881) was unable to get infection of fruits when the spores were planted on the uninjured surface. Smith

(1889), however, had no trouble in bringing about infection in ripe peaches when he sowed the spores in a drop of water on the uninjured skin. Cordley (1899) obtained similar results with plums and cherries.

Field observations indicate that infection of green plums may take place through the uninjured surface if conditions are very favorable. These cases are comparatively rare, the greatest number of infections in green fruit taking place through curculio or other wounds. It is not rare, however, to find in a rotting condition uninjured green plums which are in contact with a rotting plum that is producing spores. In the ripe fruit it is not at all uncommon to find rot due to infection through uninjured cuticle which is not in contact with that of other plums.

Cooley (1914, p. 322–323) concluded from infection experiments that "The brown-rot organism will infect fruits which are immature, even penetrating those which are not more than half-grown or those in which the pits are still soft, provided the skin is punctured." He had no trouble in infecting ripe fruits without injuring them.

In the following infection experiments, carried on to determine the relative resistance of varieties, results were obtained which differ somewhat from those of Cooley.

On June 14, 1913, five plums of each of seven varieties were put into a sterile chamber and sprayed with distilled water containing Monilia spores. The results are set forth in Table III.

TABLE III.—*Results of inoculation of green plums with Sclerotinia cinerea through uninjured cuticle*

Variety.	June 14.	June 16.	June 17.
Etopa	Plums inoculated	1 infection spot	5 infection spots on 2 plums.
Opata	do		5 fruits rotting.
Topa	do	10 infection spots	3 fruits completely rotted; 2 have 1 spot each.
A X W 15	do	15 infection spots	Spots spreading slowly.
B X W 21	do	No infection spots	2 clean; 3 one spot each; not spreading rapidly.
B X W 15	do	do	No infection spots.
Americana seedling No. 1.	do	1 through curculio wound.	4 clean; 1 completely rotted through curculio wound.

These results show very clearly that infection can take place through the injured skin of very young plums. This experiment was repeated from time to time until the plums were ripe, and at no time, if the temperature was favorable, was any difficulty encountered in obtaining infection through the uninjured surface of certain varieties.

The results given in Table III also indicate that there is considerable difference in the ease with which the varieties of plums are infected, as well as the rapidity with which the fruit rots after infection has taken

place. Is the difference in susceptibility to infection due to differences in morphological characters of the epidermis?

It has been definitely proved from time to time that the fungus has the ability to "bore" through the uninjured skin of plums and peaches. Therefore, penetration must take place either through the rather thick cuticle of the epidermal layer or through the stomata.

Morphology of the Skin and Flesh of the Plum

For a better understanding of the entrance and penetration of the fungus in the plum fruit, a knowledge of the morphology of the "skin" and underlying layers of cells is necessary.

Stomata.—The epidermis of the plum consists of a single layer of cells covered by a rather thick layer of a cutinized substance (Pl. XXXVIII, fig. 2). On the surface of this is secreted a waxy "bloom."

Stomata are present in the young fruit. In fruit about half grown changes take place in the stomata leading to the formation of lenticels.

The lenticels are formed in at least three ways:

(A) In some cases a few flat disk-shaped cells are formed parallel to the epidermis and lining the stomatal cavity. The walls of these cells appear to be of the same material as those of the deeper lying parenchyma cells (Pl. XXXVII, fig. 1). The guard cells often open wide and dry out. In other cases changes take place in the composition of the walls of about two layers of cells lining the stomatal cavity. These cells, the walls of which were originally cellulose, give the characteristic yellow staining reaction of cork with the iron-alum-hematoxylin safranin stain (Pl. XXXVII, fig. 3).

(B) In some varieties meristematic tissue develops from the parenchyma cells and produces tissue which partially (Pl. XXXVII, fig. 2) or completely fills the stomatal cavity (Pl. XXXVII, fig. 4). Occasionally a column of cells even grows out through the stomatal opening. These cells appear to be of the same nature as the hypodermal cells underlying the epidermis, in no case giving the staining reaction of cork.

(C) The lenticels, which appear as large, corky specks on the surface of ripe plums, are made of a pad of corky cells lying parallel to the epidermis. They probably develop at the stomata, splitting the guard cells apart and growing out through the opening. The details of their formation, however, have not been carefully studied in this connection, as only very few were encountered in the material examined.

Hypodermal Parenchyma.—Directly underlying the epidermis are layers of oblong cells slightly larger than and lying parallel to the epidermal layer. These make up what is commonly known as the "skin" of the plum. In some of the thick-skinned varieties there are often as many as seven or eight layers of these cells (Pl. XXXVIII, fig. 5), while in the thin-skinned forms often not more than one or two layers are present (Pl. XXXVII, fig. 1, 2, and 5).

Lying below the hypodermal layers of cells and in sharp contrast to them are the large, isodiametric cells which make up the mass of the fruit tissue (Pl. XXXVII, fig. 6). In the ripening process in those varieties which become soft these cells split apart at the middle lamella (Pl. XXXVII, fig. 5). The solution of the middle lamella apparently takes place more readily in these cells than in those of the hypodermal layers.

METHOD OF ENTRANCE OF THE FUNGUS

Two methods were used in the determination of the details of the entrance of the fungus. The first consisted of macroscopic observations on ripe or nearly ripe fruit shortly after infection had taken place. In the second method fruits of a number of varieties of plums at various stages of development were brought into the laboratory and inoculated, in some cases by a suspension of spores in water and in others by laying the plums in contact with moist mummies well covered with spores. After infection had taken place and small decayed spots had appeared, blocks of the flesh, including these spots, were killed and embedded in paraffin, according to the usual methods employed. These were later sectioned, mounted, and stained. Sections 8 to 11μ thick were found most satisfactory. Various stains were used, including Harper's short modification of the triple stain, Heidenhain's iron-alum-hematoxylin, and also a modification of this in which safranin was used. This last-named stain proved very satisfactory.

It was noticed continually, particularly in ripe or nearly ripe fruit, that when infection took place through the uninjured skin, the spot always had in its center a lenticel or "dot." These observations indicated that infection takes place, not through the cuticle, but through the lenticel in ripe or nearly ripe fruit. Further evidence was obtained on this point when sections were made of the skin from material in which the lenticels were either forming or completely formed and through which infection had taken place. It was found that the hyphæ entered between the guard cells into the stomatal cavity (Pl. XXXVIII, fig. 3, 4, and 5). In those stomata lined with corky material infection of the fruit tissue does not take place immediately, as the fungus apparently has not the power to pierce directly through the corky cells. The hyphæ continue to grow, filling up the stomatal cavity, and eventually exert enough pressure to split away the epidermis from the lenticel cells (Pl. XXXVIII, fig. 5). It is through this opening that infection takes place into the fruit tissue (Pl. XXXVIII, fig. 1 and 2).

In the young plums, before corky material has been formed, the germ tubes also enter through the stomata. After entering they come in contact with normal fruit tissue, and direct infection takes place (Pl. XXXVIII, fig. 4). In all, 44 instances of infection through stomata or lenticels were noted, and although the surface of both ripe and green plums was often

well covered with germinating spores, no instances were found in which the germ tubes gained entrance directly through the cuticle.

Further evidence that the germ tubes do not usually penetrate the cuticle was obtained when two green plums of B×W 15, a very resistant variety, were scraped lightly with a sharp knife, thereby removing the cuticle without otherwise injuring the epidermis, and were then inoculated. These, with seven others of the same variety which had not been so treated, were sprayed with distilled water containing chlamydospores and put under a bell jar. At the end of 58 hours the two plums which had been scraped showed 10 and 13 spots, respectively, but rotted very slowly from the infection points. The seven unscraped plums were at this time without infection spots, but eventually three of these showed evidences of infection.

Because of this method of infection, resistance can not be attributed entirely to morphological differences in the epidermis of the varieties. There are however, certain morphological differences in the stomata and lenticels which contribute to resistance, the nature of which will be discussed later. When once the fungus has gained entrance the plums always rot more or less rapidly, depending upon the variety.

FIELD OBSERVATIONS

It is apparent from the facts given that the small amount of rot found in the orchard on green plums is not due to any greater resistance to infection which the green fruit may possess over ripe fruit. Nevertheless, the brown-rot in the orchard causes the greatest damage as a ripe-rot rather than as a green-rot.

It is a fact of considerable importance that it is not until the plums are ripe and begin to soften slightly that the fungus does its greatest damage as a ripe-rot. This is due probably to two reasons. The first is that there are greater possibilities of infection at this time. Field observations show that green plums will rot on the trees, owing usually to infection through curculio or other wounds, and that the rot will spread from one to another where they are in contact. Thus the number of rotted fruits and hence of infection sources to the ripe fruit is gradually increasing. Although there are other methods of infection, the largest number in ripe fruit is due directly or indirectly to contact with rotten green plums. It is very common in the field to find large groups of plums on a tree completely rotted, while other groups on the same tree are entirely free from rot. In these groups it is nearly always possible to trace the original source of infection back to one plum which has in most cases been infected through a wound of some kind while still green.

Another source of infection, more common in ripe or nearly ripe fruits than in green fruits, is direct infection from spore suspensions in water,

due probably to the greater number of spores being produced. This is not of considerable importance, however, except under extremely favorable weather conditions, when it may be the cause of a great deal of damage to fruits (Smith, 1889). A source of infection, common in completely ripened fruits and not common to green fruits, is through wounds caused by the cracking of the plums. This cracking is due either to excessive rainfall after a dry period, causing a rapid increase in turgor with the consequent splitting of the fruit, or to water remaining between plums which are in contact. This effect was also noted when ripe plums kept in a moist chamber cracked where they were in contact with the glass if water was present.

The second reason for the ripe-rot effect is the fact that the ripe fruit of some varieties is much more susceptible to rot after infection takes place than the green ones (see p. 388).

VARIETAL RESISTANCE OF PLUMS TO THE FUNGUS

That plums and peaches vary in their resistance to brown-rot has been noted from time to time. This power of resistance has been ascribed to various causes, such as a thick skin in certain varieties of resistant plums, a small amount of down on resistant peaches, and late ripening of some varieties, with consequent avoidance of the disease because of temperature conditions.

During the summer of 1913 attempts were made to determine whether definite differences in resistance to the brown-rot fungus really exist in plum varieties. Inoculation tests were started as early as June 14, when the plums were about one-third grown, and carried through on some varieties until maturity. Infection was brought about at first by spraying the plums with distilled water containing the spores. Later, a more effective method was found to be that of placing the plums in contact with moistened mummies well covered with spores. In both cases the experiments were carried on under bell jars in the laboratory.

RELATIVE RESISTANCE OF VARIETIES

Table IV shows the relative resistance of varieties as determined by the inoculation of 262 plums through uninjured skin and the subsequent rotting of the fruits.

The skin and flesh descriptions, except where indicated, were taken from a table prepared by Dr. M. J. Dorsey, of the Minnesota Experiment Station, in a study of "fruit characters" in hybrid plums, prepared independently of the investigations on resistance. The descriptions of varieties indicated by an asterisk (*) were made by the writer.

TABLE IV.—*Texture of flesh and skin, ripening date, and relative resistance of varieties of plums to Sclerotinia cinerea*

Variety.	Date of ripening.	Texture of flesh.	Texture of skin.	Thickness of skin.	Relative susceptibility.[a]
A × W 2	Aug. 25	Medium firm, tender	Medium	Medium	+
A × W 11	Aug. 19	Firm, medium tender	Tough	Thin	++
A × W 12					+++
A × W 15	Sept. 2	Firm, tender	Tough	Medium	++
A × W 17	Aug. 18	Tender	Medium	do	
A × W 18	Sept. 2	Medium firm, tender	Tough	Medium+	+
B × W 1	Aug. 31	Soft, tender	Medium	Medium	++
B × W 2	Aug. 31	do	Tender	Thin	+++
B × W 4	Sept. 2	do	Medium	Medium	++
B × W 5	Sept. 2	Medium firm, tender	do	Medium+	++
B × W 6	Aug. 22	Firm, tender	Tough	Medium	++
B × W 9	Aug. 31	do	do	do	+
B × W 12	Aug. 18	do	do	do	++
B × W 15	Sept. 2	Very firm, medium tender.	Medium	Thick	+
B × W 16	Aug. 27	Soft, tender	do	do	++
B × W 21	Aug. 19	Firm, tender	Tough	do	++
*S. D. No. 1	Aug. 15	Soft, tender	Tender	Thin	++++
*S. D. No. 2	Aug. 15	Firm, tender	Medium	Medium	+
*S. D. No. 3	Aug. 15	do	do	do	+
Burbank	Aug. 17	Soft, tender	Tough	do	+++
Wolf	Sept. 1	Medium firm, tender	Medium	Thick	++
*Ocheeda			Tough	Medium	++
*Harrison			do	do	++
*Surprise			Medium	Thick	+++
*Hammer			do	Medium	+++
*Newman			do	do	+++
*Manitoba No. 1.			Tender	do	++++
*Americana seedling No. 1.		Firm, tough	Tough	Thick	+
*Americana seedling No. 2.			do	Thin	++++
Etopa		Soft, tender	Tender	do	++++
Opata	Aug. 17	do	Medium	do	+++
Okiya	Aug. 18	do	Tender	do	++++
Wakapa	Aug. 18	do	do	do	++++
Compass	Aug. 15	do	do	do	++++
Sand cherry	Aug. 10	do	do	do	++++
*Reagan	Sept.	Very firm, medium tender.	Tough	Thick	+

[a] + Indicates least relative susceptibility; ++++ indicates greatest relative susceptibility.

The results show striking differences in resistance of the several varieties to infection. In the case of very susceptible varieties, as the Compass and sand cherry, it is always very easy to get a large number of infection spots. In the case of a very resistant variety, such as B × W15, it is often very hard to cause infection. In one trial, begun on July 8, 1913, in which green plums, about three-quarters grown,

were inoculated by contact with mummies in a moist chamber, the following results were noted after 27 hours:

Variety.	Number of plums.	Points of contact.	Number of infection spots.
B × W10	1	1	Many.
B × W2	1	1	Do.
Burbank	1	1	20.
B × W15	4	6	None.
Topa	1	1	1.
Opata	1	1	Many.

Another trial with B×W15, directly following this and carried on under the same conditions, showed a few infection spots in three out of five contact points, indicating that in some cases the fungus can enter these resistant plums. A number of other experiments, comparing the relative resistance to infection of B×W15 with that of other varieties, showed results comparable to those given above.

Soon after infection takes place a small decayed spot appears on the surface of the plum. These spots increase in size rapidly in the susceptible varieties and soon completely cover the plum. This often requires not longer than 24 hours after infection has taken place. On the resistant forms, however, the spots increase in size slowly, sometimes taking several days before they entirely cover the plum. The rapidly rotting plums take on the characteristic brown color of rotten fruit; but the slower rotting varieties often become dark blue and when completely rotted become black.

Usually when the susceptible varieties are one-half to three-quarters rotted, they begin producing tufts of chlamydospores over the rotted area. On the sand cherry and some of the sand-cherry hybrids, which are very susceptible, the spore tufts are usually large and numerous (Pl. XXXVIII, fig. 9). Varieties such as B×W21, which appear intermediate in the rapidity with which they rot, usually produce spore tufts, but they are nearly always smaller and less numerous than those on the susceptible varieties (Pl. XXXVIII, fig. 7 and 8). In the case of the most resistant varieties it is seldom that spores are produced if the skin has not been broken. If the plum has been wounded, spores are usually produced through the wound (Pl. XXXVIII, fig. 6). Under particularly favorable conditions pustules may appear through the uninjured skin, in which case they are usually small, and few in number.

RELATION OF SKIN THICKNESS TO RESISTANCE

In order to determine the part played by thickness of skin in resistance, inoculations were made by cutting off a small piece of skin and planting

the spores on this freshly cut surface of the plum in a drop of water. The plums were kept in a moist chamber. The same relative differences in rapidity of rotting were noted in these cases as when the infection took place through the uninjured skin, indicating that mere thickness of skin is not the deciding factor in resistance, as the cells underlying the skin show the same relative resisting powers.

However, it will be seen by referring to Table IV that the varieties which are the most susceptible are the thin-skinned, tender-fleshed ones, while the more resistant varieties are thick-skinned and of a firmer, tougher texture. An examination of prepared slides of the skin of the different varieties confirms these observations, in that all of the very susceptible varieties have a thin skin (Pl. XXXVII, fig. 4), consisting of one or two layers of cells besides the epidermis; while the resistant varieties all have a very thick skin (Pl. XXXVIII, fig. 4), consisting of from five to eight layers of cells. The varieties appearing to be intermediate in resistance have skins varying in thickness, but in all cases examined they are thicker than the susceptible forms. It would seem, then, that there is a rather close correlation between skin thickness and resistance to the brown-rot fungus.

RELATION OF STOMATA AND LENTICELS TO RESISTANCE

In studying the method of infection, a comparison of the stomata and lenticels of the different varieties revealed some interesting and important facts relating to resistance. The lenticels described above, in which no change other than the production of a few flat cells lining the cavity (Pl. XXXVII, fig. 1) took place, were found only in the thin-skinned varieties, as Gold and some of the sand-cherry hybrids. Those in which the lining cells became corky (Pl. XXXVII, fig. 3) were found in the thicker skinned varieties.

In two of the most resistant varieties, B×W15 and A×W9, the formation of lenticels, due to filling of the stomatal cavity with parenchyma cells, was very common (Pl. XXXVII, fig. 4). This condition was not entirely confined to these varieties, as instances were found in many others of the thick-skinned varieties and also in such a thin-skinned variety as Gold (Pl. XXXVII, fig. 2 and 4), where, however, only a few cells were formed that did not in any case completely fill the cavity (Pl. XXXVII, fig. 2).

That the complete plugging of the stomata is a factor in resistance is shown by the fact that many instances were noticed in which these stomata were completely covered by germinating spores, with no resulting infection. It did take place, however, through stomata the cavities of which were only partially filled with these cells and also through those in which only the corky tissue was present (Pl. XXXVIII, fig. 1, 2, and 5). This may explain why it was possible to obtain so few infections in A×W9 and B×W15, even when their surfaces were covered with germinating spores.

PHYSIOLOGICAL RELATION OF FUNGUS TO HOST

That resistance is not entirely due to the partial inability of the fungus to gain entrance to the tissues of the resistance forms is shown by the difference in rapidity of rotting after infection has taken place. A study of the further penetration of the fungus in the resistant and susceptible forms was therefore undertaken.

Previous investigators do not agree as to the manner in which the fungus penetrates the host tissues, some holding that it penetrates the cell walls wherever it comes in contact with them and that it shows no particular affinity for the middle lamella (Cooley, 1914), while others hold that the fungus follows the middle lamella and may or may not split it completely (Schellenberg, 1908; Bruschi, 1912).

The method used in the present study of the relation between the host and the fungus cells was the same as that used in the determination of the method of infection—i. e., a study of prepared slides of infected plum and apple tissue. The stains already mentioned were used. The material consisted of small blocks of plum and apple tissue cut from the edge of the rotting spots and also blocks cut from plums which had been infected within 12 to 30 hours of the time of killing. For this study of the penetration of the fungus, over 220 slides were prepared from material collected from 17 varieties of plums and 4 varieties of apples. In 80 of these slides the fungus hyphæ were clearly differentiated from the host tissue.

PENETRATION

In all cases the fungus shows a very strong affinity for the middle lamella (Pl. XXXVIII, fig. 2, and XXXIX, fig. 1, 2, 5, and 6). No instances were found where the hyphæ had actually pierced the cell walls and entered the cell cavity, so that it seems certain that the hyphæ of *S. cinerea* are unable to penetrate the cell walls of the plum and apple fruits. No record has come to notice of other investigators having extracted from the brown-rot fungus a cellulose-splitting enzym which has the power of dissolving the plum cell walls. Furthermore, that such an enzym is not produced by the fungus in the host tissues is clearly demonstrated by the fact that in completely rotted plum tissue (Pl. XXXIX, fig. 5) and in sclerotia which have been buried in the ground for over 18 months and have produced apothecia, the cell walls are still intact.

From the appearance of the infected tissue it is evident that the fungous hyphæ secrete a substance which splits out the middle lamella slightly in advance of its penetration through the tissue (Pl. XXXIX, fig. 1, 2, 3, 5, and 6). Eventually the middle lamella is completely dissolved, leaving the cells in the rotted area entirely free from one another. Instances comparable to those illustrated were found in nearly all of the slides examined.

The killing of the host cells, so far as is revealed by the microscopical examination, seems due principally to a modification of the osmotic relations of the cells as a result of the disappearance of the middle lamella and to much of the liquid contents of the cells being withdrawn by the fungus to be used in its development. In the plum the chloroplasts and chromoplasts contained in the cells lying directly under the epidermis appeared not to be disintegrating in those cells which had not so collapsed as to make observation impossible. The cytoplasm of the deeper-lying cells was very scant, but showed evidences of plasmolysis, often unmistakably in advance of the penetration of the hyphæ (Pl. XXXIX, fig. 3).

MIDDLE-LAMELLA SOLVENT

The nature of the substance secreted is not at all clear. From the effect on the host tissue it would appear that the middle-lamella-dissolving enzym pectinase was produced, but attempts to isolate it were without success.

Juice was pressed from rotten portions of apples and loquats (*Eriobotrya japonica*) infected with the brown-rot fungus. This was filtered under sterile conditions, in some cases through coarse, and in others fine filter paper. Slices of healthy apple and loquat fruits were partially immersed in the liquid, but showed no softening effect in any case after several days. Further trials with a method to be described later, used in separating pectinase from *Penicillium expansum*, also gave negative results with *S. cinerea*.

In another case a partially rotted apple plug was put into a test tube on cotton above commercial formalin so that the plug did not come in contact with the liquid. It was thought that the fungus would be killed by the fumes, but that if a pectinase were present it would continue to rot the tissue. No further rotting took place, and at the end of five days the tissue, unaffected at the beginning, was still firm and of normal color.

An attempt was made to isolate the enzym pectinase from a culture of *S. cinerea*, 86 days old, on apple cider. The method used was that described by Pringsheim (1910), which consists, in brief, of thorough drying of the material with acetone, followed by pulverization of the dried material and extraction of the enzym with a small quantity of water. On May 8, 1915, succulent twigs of B × W21 plum, sand cherry, and pear (*Pyrus betulifolia*) were partially immersed in the liquid extract in test tubes; also pieces of ripe apple the flesh of which was slightly mealy, and pieces of young peaches, one-quarter grown, were entirely immersed. The tubes were placed in a constant-temperature oven at 35° C. Checks were run, using water in place of the extract.

After 24 and 48 hours the plum, pear, and sand-cherry twigs showed no effects from the treatment other than a slight wilting. The tissues were not softened. The blocks of green-peach fruit showed no softening. After 15 hours the apple plug had softened slightly over the surface, but

was still firm in the center. After 48 hours it had softened completely. A portion not immersed in the liquid, but which came in contact with it at one point, was softening from this point and becoming discolored. The checks in water remained firm and were not discolored.

Although the effect of the extract on the apple tissue appeared to be that of a pectinase, it can hardly be concluded that this enzym was present, as the fruit used was overripe and slightly mealy, and could very easily have been broken down by other solvents contained in the extract.

DeBary (1886) considered the possibility of oxalic acid being the toxic substance produced by *S. libertiana*, because he found the hyphæ often coated with crystals of it; however, he later discarded this notion for the reason that solutions of oxalic acid did not give the same effect as the fungus. Smith (1902) extracted a substance from *Botrytis cinerea*, which, whether boiled or unboiled, caused a rot of the host tissue identical with that caused by the fungus. He concluded it was not an enzym, but that the effect might be due to oxalic acid, which he found to be present in quantities often as high as 2 per cent. Peltier (1912) confirmed the results regarding this action of the extract, but was unable to detect the presence of oxalic acid, even in old cultures.

The possibility of oxalic acid being the toxic substance of *S. cinerea* was considered, as Cooley has demonstrated that it is produced in appreciable amounts in cultures of *S. cinerea* on plum and peach juice, and in peaches which had been rotted by the fungus. In order to determine the effect of oxalic acid on vegetable tissue, small blocks of onion, potato, tomato, dahlia, radish, coleus (young shoot), tomatoes (young shoot), loquat (fruit), canna (bulb), oxalis (petiole), geranium (young shoot), and apple were immersed in 0.015, 0.062, 0.125 per cent solutions of oxalic acid and the effect noted at the end of 24 and 48 hours. In all of the solutions the apple, loquat, and oxalis softened, while in the 0.125 per cent solution only the onion and tomato softened slightly. The potato did not soften even in 0.25 per cent solution. In all cases bleaching occurred. An examination of the different tissues showed that the softening was due to the solution of the middle lamella.

The fact that oxalic acid even in such dilute solutions readily softened the tissues of the apple and loquat, upon both of which the brown-rot grows readily, might indicate that the oxalic acid was the toxic substance, but the bleaching effect produced by the acid and the fact that when used even as strong as 0.25 per cent it had no effect on potato, upon which the fungus also grows readily, would seem to indicate that this acid is not the sole toxic substance produced.

COMPARISON OF FIRM-ROT AND SOFT-ROT

Cooley (1914) pointed out the very interesting fact that, although *P. expansum* and *S. cinerea* apparently acted differently on their hosts, the one producing a soft-rot of fruits and the other a firm-rot, in culture

they gave identical results when grown on media containing cellulose from various sources, or calcium pectinate. They were able in certain cases to hydrolyze the cellulose, but showed no dissolving action on calcium pectinate.

In order to determine the difference between a soft-rot and a firm-rot caused by fungi which physiologically were acting alike in culture, apples rotting from *P. expansum* were examined. A smear of the rotted tissue revealed the fact that the host cells were entirely separated from one another, but that the walls were apparently intact. A few very small hyphæ could be seen, seeming to be entirely intercellular. Further examination of prepared slides of material, taken both from the oldest portion of a spot 3 inches in diameter and from the edge of the rotting spot, confirmed the above observations. The middle lamella was completely split out between all of the cells in the rotted area, and the cellulose walls were entirely intact. The few very small hyphæ that were found were intercellular (Pl. XXXIX, fig. 4). So far as could be seen, the two fungi, *S. cinerea* and *P. expansum*, act in exactly the same way on the host tissue. The reason for one causing a firm-rot and the other a soft-rot is not, then, due to any differences in physiological action, but appears to be merely mechanical, due to the fact that *S. cinerea* completely fills the intercellular space produced by the collapse of the cells (Pl. XXXIX, fig. 5), with very large hyphæ, while *P. expansum* produces few small hyphæ, which give little support to the host tissues, and, as a consequence, they collapse as the rot proceeds (Pl. XXXIX, fig. 4).

The complete solution of the middle lamella in tissue rotted by *P. expansum* would seem to indicate the presence of a middle-lamella-dissolving enzym. To test this, squares of very fine-grained filter paper were laid on blocks of apple and small portions of flesh from the edge of the rotting spot were laid on the filter papers. All precautions were observed, in order to keep the materials sterile. It was thought that if a pectinase were present it would filter through the paper and cause a soft-rot of the fruit. The papers bearing the rotted flesh were removed after 3½ hours. In four cases out of seven, infection took place through the filter paper and the normal soft-rot followed, while in the three other cases the blocks became soft and translucent at the end of two days, but showed no signs of infection. A microscopic examination showed the cells to be separated from one another, owing to the complete solution of the middle lamella. The checks remained firm. A small portion of the tissue, which rotted in the absence of hyphæ, when transferred to the checks caused them to rot rapidly. This and the fact that in the typical rot spots the middle lamella is completely dissolved in the presence of very few hyphæ would indicate that *P. expansum* secretes a very active middle-lamella-dissolving enzym, pectinase.

RESISTANT AND SUSCEPTIBLE VARIETIES

The fungus hyphæ of *S. cinerea* in both resistant and susceptible fruits show practically no constant differences. In both cases they are large and densely protoplasmic over their entire length. In a few instances hyphæ in resistant forms appeared more knotted and irregular than in susceptible ones, but this could be explained in those cases by mechanical pressure of the small cells of the hypodermal layer, which in the resistant plums appear to be less easily collapsed than in the susceptible varieties. Considerable difference, however, could be noticed in the rapidity with which the hyphæ developed in the two forms. The hyphæ in the susceptible varieties usually completely filled the intercellular spaces as the rot spread, while in the resistant ones fewer hyphæ were produced. A few instances were noticed in resistant varieties of cells lying completely or nearly completely surrounded by hyphæ from which the middle lamella had not been dissolved. This and the fact that in these forms the middle lamella seldom appeared to be dissolved out far ahead of the penetration of the fungus lead to the conclusion that this partial resistance is due to the inability of the toxic material secreted to dissolve the middle lamella as rapidly in the resistant as in the more susceptible varieties, owing possibly to very slight differences in its composition.

That there is an actual difference in the composition of the middle lamella material seems fairly certain. It is well recognized that varieties of plums, apples, and other fruits and vegetables vary greatly in the time required for cooking. Some remain firm after a long period of boiling, while others soften and become mushy after very short heating. An examination of boiled-apple tissue which had become soft revealed the interesting fact that the softening was due in part to a separation of the cells as a result of the middle lamella having been dissolved. The cell walls appeared not to be ruptured at all. In those varities which do not become soft on boiling it is assumed that the middle lamella material is less soluble and therefore is probably of a slightly different chemical composition. It is recognized, of course, that the dissolving action of the fungus upon the pectic substances and solution by hot water are entirely different processes and, therefore, resistance to the fungus and firmness after cooking may or may not be correlated.

In view of the fact that eventually in both resistant and susceptible forms the middle lamella is completely dissolved, the difference in sporulation (Pl. XXXVIII, fig. 6, 7, 8, and 9), as described above, could hardly be explained by variations in middle lamella composition, but rather points to a small amount of some toxic substance being produced either by the host cells or fungus hyphæ, which is not enough to completely stop the growth of the fungus, but merely to retard slightly its normal functioning.

TOXICITY OF ORGANIC ACIDS TO THE FUNGUS

In a series of tests carried on by the writer to determine the relative toxicities of the fruit acids to *S. cinerea*, results were obtained with regard to oxalic acid which may throw some light on the cause of these differences in sporulation. Hanging-drop cultures containing large numbers of the chlamydospores in suspension in solutions of oxalic, tannic, gallic, tartaric (inactive), malic, and citric acids were used. In all of the tests the oxalic-acid solutions were found to be by far the most toxic. As has been noted, Cooley (1914) found this acid to be produced in appreciable quantities by the fungus in culture. In view of this, it is very possible that in the slow development of the fungus in the resistant fruits enough oxalic acid is produced by the hyphæ to actually become toxic to them, resulting in the production of few or no spore tufts.

RIPE-ROT

The discussion of the penetration of the fungus thus far has had special reference to green and ripening plums, but not to those plums which have begun to soften slightly as a result of the ripening process. It is when the plums begin to soften that the fungus works the greatest havoc, and it is then that variations in resistance are most noticeable in the orchard.

Cook and Taubenhaus (1912) were able to demonstrate a positive correlation between the decrease in the oxidizing-enzym content of the fruits of many plants, due both to maturing and to removal of the fruit from the plant, and a decrease in their resistance to certain diseases. They could show no correlation between acid content of apples and pears and resistance to disease. Cooley (1914) was able to confirm these latter results in the plum, finding that as the plums matured the acid content increased until it reached its maximum at the time of ripening of the fruit, which was also the period of greatest susceptibility to the brown-rot fungus. As acidity will not explain the decrease in resistance of plums to the rot on ripening, can it be explained by a decrease in the oxidizing-enzym content of the plums?

Ripe fruits of the Reagan plum, which is a resistant variety, were sent to this Station from New York on October 22, 1914. On November 7 they were inoculated with the brown-rot, both by spraying on spores and by laying the plums in contact with moistened mummies. By this time the oxidizing enzym should have entirely disappeared, owing both to ripening and to removal from the tree. In spite of this, the plums were found to be still very resistant both to infection and to rot after infection occurred. It is evident then that resistance can not be due in this case to the presence of the oxidizing enzym.

Material of these plums was sectioned, and it was found that in the healthy tissue of these very ripe plums the middle lamella was still

present (Pl. XXXVII, fig. 6). The plums at the time of preserving the material (Nov. 7, 1914) were firm. An examination of the healthy tissue of ripe susceptible varieties revealed the fact that the middle lamella in these was completely dissolved (Pl. XXXVII, fig. 5). These plums were soft when the material was fixed. That the pectic-acid compounds change to pectin in the ripening fruit is a well-known fact. In view of the fact that the brown-rot can only spread after the middle lamella has been dissolved, the reason for the increase in susceptibility on ripening in those varieties which become soft as a result of the normal loss of the middle lamella owing to ripening is readily seen.

The reduced possibilities of infection owing to the plugging of many of the stomata, the causes of which have already been explained, and the persistence of the middle lamella after ripening, as shown by the fact that the fruits remain firm, explain the resistance to brown-rot of such varieties as Reagan, B×W15, B×W9, S. D. Nos. 2 and 3, and Americana Seedling No. 1.

RELATION OF TANNIN CONTENT OF THE HOST TO RESISTANCE

A great deal of attention is being given to the relation between chemical substances within the host cell and resistance. The work of Comes (1913) on the correlation between the increased acid content in wheat plants and rust resistance has been mentioned. Cook and Taubenhaus (1911) were able to show that tannin, a very common product in plants, was toxic in varying degrees to many fungi in culture and considered that it might be a very important factor in resistance. Bassett and Thompson (1911) showed that apples and pears contain an oxidizing enzym capable of producing from gallic acid a tannin-like substance having the power of precipitating protein from solution. They found this product to be toxic to "a fungus." The juices of green apples, pears, and walnut hulls (unboiled) produced a substance which on standing precipitated soluble protein from the juice. They considered this to be a tannin-like substance and to be controlled by the oxidizing enzym.

If the tannins disappear on the ripening of the fruit, as is generally supposed, we may have an explanation of the greater susceptibility of some fruits to disease on ripening. The evidence of the disappearance of tannin on ripening, however, is not at all conclusive. One of the most striking instances of its apparent disappearance is that of the persimmon (*Diospyros virginiana*), the green fruits of which are very astringent, while the ripe, soft fruits are not at all astringent. Gore (1911), however, showed that the tannin did not disappear, but was inclosed in sacs which broke readily in green fruits in contact with saliva, but were not affected in the ripe fruit. Similar structures have been observed in the carob-bean pod (*Ceratonia siliqua*) and in the date fruit. Bassett and Thompson (1911) demonstrated that "apples that had fallen from

the tree showed about twice as much tannin as those freshly plucked." It is a matter of common observation that some plums, especially the sand cherry, contain considerable amounts of an astringent substance, probably tannin, even when dead ripe. It is not altogether clear, therefore, that the disappearance of the tannin on ripening is a cause of the increased susceptibility of ripe fruits to rot.

There is still the possibility that differences in resistance of varieties may be due to unequal tannin content. In order to determine this point, tannin determinations were made of the fruit of 11 varieties of plums. The method used was Proctor's modification of Lowenthal's method as described by Leach (1913, p. 370). The results given in Table V are for tannin substances calculated as gallotannic acid. The determinations were made on fruit which had been picked 14 hours, except in the case of the sand cherry and Compass, which were made directly after picking.

TABLE V.—*Tannin content of ripe and green plums on August 6, 1915*

Variety.	Condition.	Date of ripening.	Percentage of tannin in pulp.	Percentage of tannin in dry matter.	Percentage of dry matter.	Relative susceptibility.
Sand cherry	Ripe	Aug. 1	2.087	15.081	13.84	++++
131 × (sand-cherry hybrid)	do		.234	1.483	15.75	++++
Compass × pin cherry	do		.338	2.388	14.17	++++
Sapa	Turning	Aug. 17	.362	3.367	10.75	++++
Compass	Green	Aug. 15	.483	4.229	11.42	++++
A × W12	do		.482	3.418	14.10	+++
Opata	Turning	Aug. 17	.733	4.618	15.87	+++
Burbank	Green	do	.185	1.516	12.20	+++
B × W21	do	Aug. 19	.773	5.777	13.38	++
A × W15	do	Sept. 2	1.131	9.520	11.88	++
Americana Seedling No. 1.	do		.665	3.873	17.17	+

The relative-susceptibility determinations were made at the same time as the tannin determinations and are confirmed by previous tests on some of the varieties and by field observations on all of them.

It is readily seen that very little relationship exists between tannin content and resistance to the brown-rot fungus. Even though a correlation could be shown between tannin content and resistance, it still remains to be proved that the tannin is an actual factor in resistance, since the following facts indicate that it does not come into direct contact with the fungus hyphæ. The hyphæ are apparently always intercellular, and according to Haas and Hill (1913, p. 192)—

> In the cell the tannin occurs in solution in the cell sap, and since tannin forms a precipitate with albuminous matter it follows that the layer of protoplasm around the tannin vesicles must be impermeable to it; if this were not so the protoplasm would be tanned on the production of tannin.

CONCLUSIONS

(1) The brown-rot fungus in Minnesota seems to be identical with that found in other parts of this country and with *Sclerotinia cinerea* of Europe. Chlamydospore tufts vary in color from gray to bright ocher. For the production of the ascus stage the sclerotium apparently must be buried in the ground for two winters. Mummies which have hung on the trees for one year are still capable of producing apothecia.

(2) Infection may take place through the uninjured skin at any time during the development of the plum fruit. The hyphæ enter through the stomata and lenticels. Varieties show great differences in resistance to infection, owing to the production of parenchymatous plugs which fill the stomatal cavity and to lenticels made up of layers of corky cells through which the hyphæ are unable to penetrate. Corky cells lining the stomatal cavity merely delay infection.

(3) Varieties show variations in resistance to rot after the hyphæ have gained entrance. Resistance is apparently correlated with (a) a thick skin; (b) the production of parenchymatous plugs which fill the stomatal cavity; (c) the production of corky walls in the lining cells of the stomatal cavity; and (d) firmness of fruit after ripening. There seems to be no relationship between oxidase content of the fruit and resistance or between tannin content and resistance.

(4) Brown-rot is essentially a ripe-rot, affecting the plums most noticeably as soon as they begin to soften slightly as a result of ripening. Varieties which are resistant remain firm on ripening. Softening during ripening is due to the solution of the middle lamella.

(5) The hyphæ of *S. cinerea* in the tissue of plum and apple fruit are entirely intercellular. The middle lamella is dissolved slightly in advance of the penetration of the hyphæ. The absence of the middle lamella in fruits which have softened owing to ripening explains the greatly increased spread of the disease at ripening time. Attempts to demonstrate the presence of the middle-lamella-dissolving enzym, pectinase, in rotting fruits or to extract it from a culture of the brown-rot fungus on apple cider proved futile.

(6) The rot caused by *S. cinerea* is a firm-rot due to the mechanical support of the hyphæ which completely fill the intercellular spaces left by the collapse of the host cell walls. *Penicillium expansum* produces a soft-rot, because of the fact that few hyphæ are produced and, therefore, little mechanical support is given to the rotted tissue, which as a consequence collapses as the rot proceeds. The hyphæ of *P. expansum* are intercellular and produce a substance which dissolves the middle lamella even in the absence of the fungus hyphæ.

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PLATE XXXVII

Fig. 1.—Lenticel in ripe fruit of Sapa plum. The walls of the cells lining the cavity give the staining reaction of cellulose. × 400.

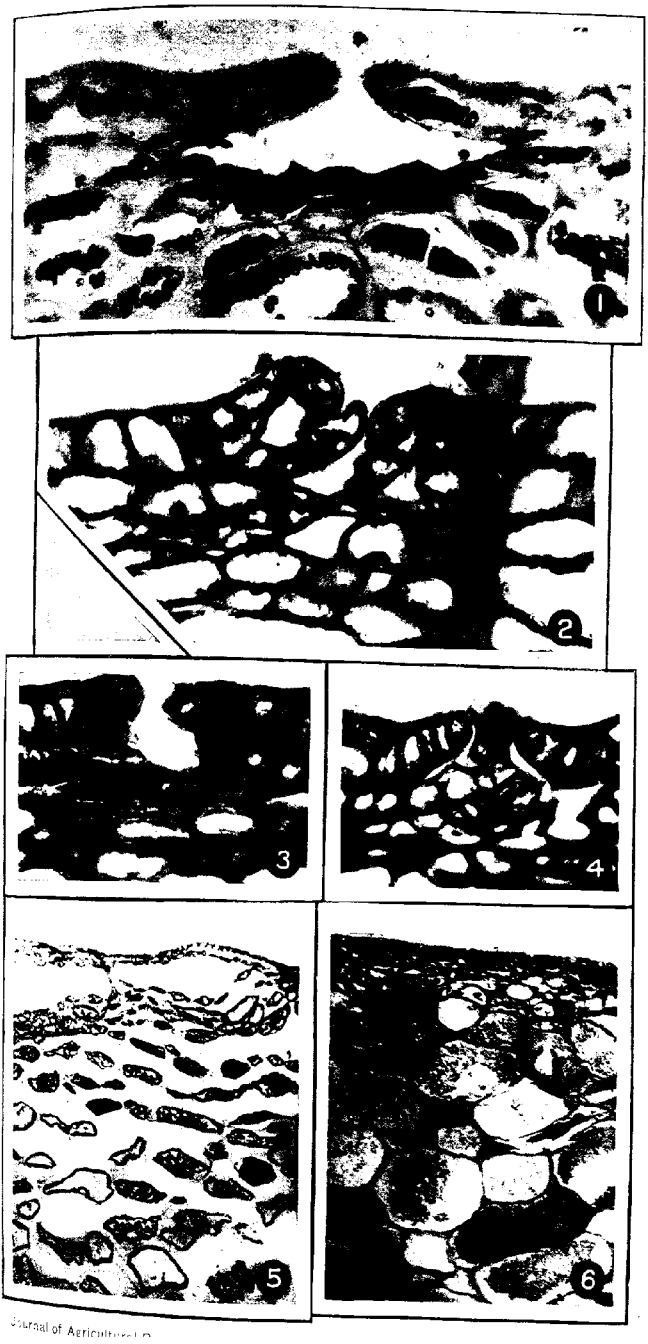
Fig. 2.—Lenticel in ripe fruit of Gold plum partially filled with parenchymatous cells. Infection may take place through a lenticel of this type. × 400.

Fig. 3.—Lenticel in green Burkank plum. The cell walls lining the cavity give the staining reaction of cork. Infection may take place through a lenticel of this type, but only in the manner shown in Plate XXXVIII, figures 1, 3, and 5. × 400.

Fig. 4.—Lenticel in green fruit of B × W 21 completely filled with parenchymatous tissue. Infection can not take place through a lenticel of this type. × 400.

Fig. 5.—Ripe healthy tissue of Sapa plum, showing middle lamella completely dissolved out owing to ripening process. This is the condition found in the ripe fruits of the susceptible varieties. × 60.

Fig. 6.—Ripe healthy tissue of Reagan plum two weeks after picking. The middle lamella is still intact. This is the condition found in the ripe fruit of resistant varieties. × 60.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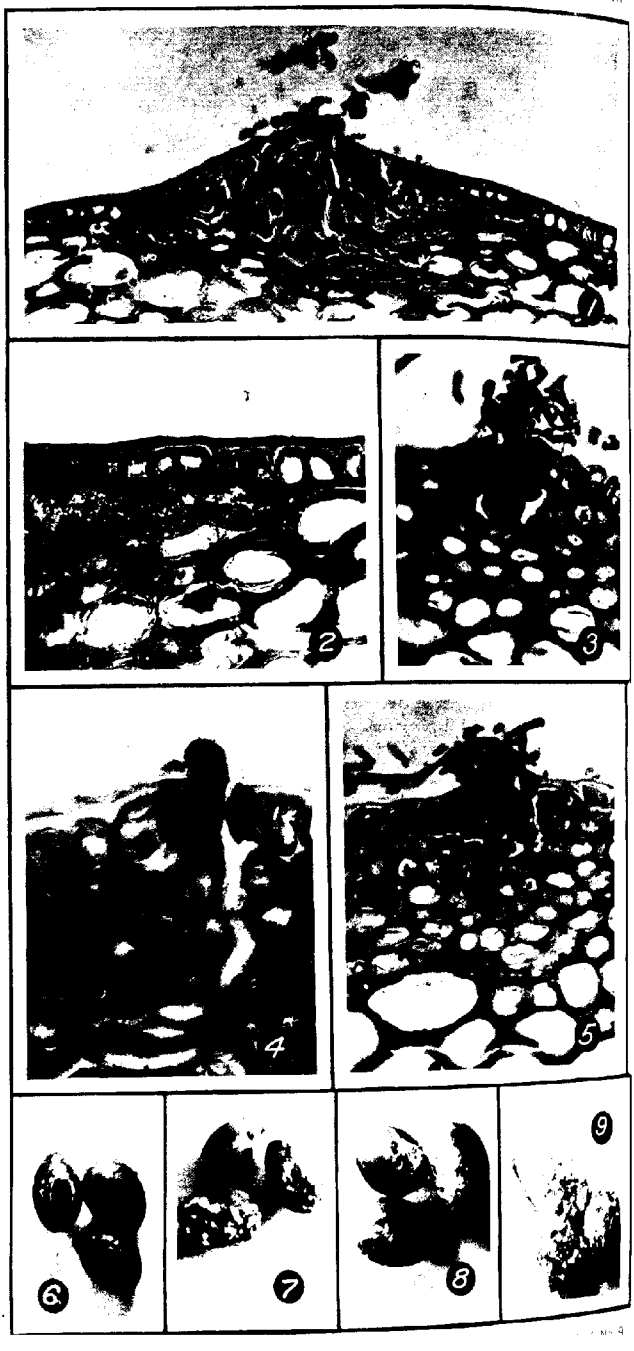
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PLATE XXXVIII

Fig. 1.—Infection through a lenticel of Burbank plum the cavity of which is lined with corky-walled cells. The hyphæ are incapable of dissolving the middle lamella between these cells, but apparently exert enough pressure to split the epidermis away from the underlying cells, thereby allowing the hyphæ to enter the fruit tissue. × 216.

Fig. 2.—Left side of figure 1 in detail, showing hyphæ entering the fruit tissue after the epidermis has been raised by the growth of the hyphæ in the stomatal cavity. × 400.

Fig. 3.—Infection through a lenticel in B × W_4. The hyphæ swell on entering, filling up the stomatal cavity. × 200.

Fig. 4.—Infection through a stoma in a young green fruit of *Prunus americana* seedling No. 1, in which no corky walls have yet been formed. × 400.

Fig. 5.—Infection through a lenticel of the same type as is shown in figures 1 and 3. The hyphæ have filled the stomatal cavity and are raising the epidermis from the underlying cells. The hyphæ can enter the fruit tissue through the split thus formed. × 200.

Fig. 6.—Half-grown fruits of B × W_{15} completely rotted through wound inoculations. Only very few spore tufts are being produced. This is a resistant variety.

Fig. 7.—Half-grown fruits of B × W_{21} completely rotted through wound inoculations. This variety is intermediate in degree of resistance.

Fig. 8.—Half-grown fruits of A × W_{15} completely rotted through wound inoculations. This variety is intermediate in degree of resistance.

Fig. 9.—Half-grown fruits of Etopa plum completely rotted through wound inoculations. The plums are completely covered with large spore tufts. This is a very susceptible variety.

PLATE XXXIX

Fig. 1.—A rotting area in an overripe fruit of S. D. No. 3. In the healthy portion at the right the middle lamella is still intact, while in the rotted portion the cells are free. This is a resistant variety. × 216.

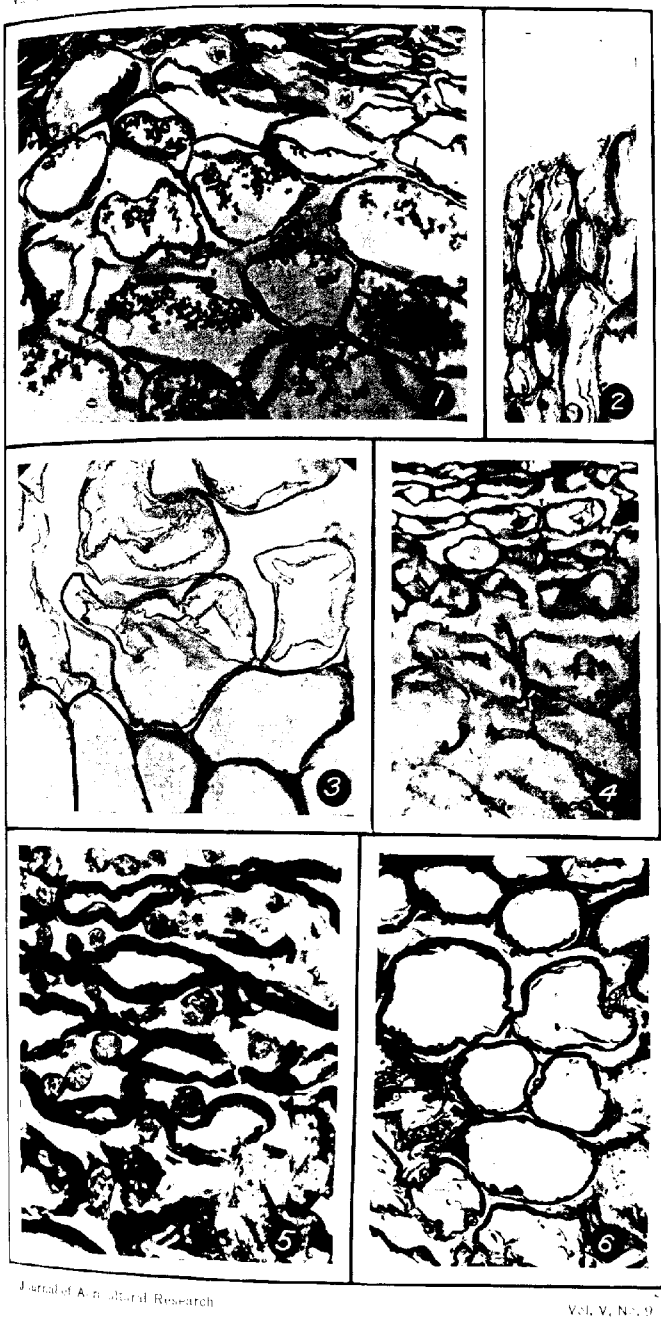
Fig. 2.—Tip of hypha in Opata plum. The middle lamella is being split slightly ahead of the hyphæ. This is apparently not due to mechanical pressure, as the walls in contact with it are collapsed. × 200.

Fig. 3.—The edge of a rotting spot in a green fruit of Opata plum. The middle lamella is dissolved in advance of the penetration of the hypha. This is a susceptible variety. × 216.

Fig. 4.—Tissue of apple infected with *Penicillium expansum*. A short piece of hyphæ may be seen in the center of the figure. The middle lamella is completely dissolved. × 156.

Fig. 5.—Cross sections of hyphæ in tissue of Opata plum 18 hours after inoculation. The dark areas are collapsing cell walls. The hyphæ are entirely intercellular. × 400.

Fig. 6.—Portion of the rotted area of an Opata plum 18 hours after inoculation. Although only few hyphæ are present, the middle lamella is completely dissolved. × 200.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FREQUENCY OF OCCURRENCE OF TUMORS IN THE DOMESTIC FOWL[1]

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The work of Rous, Murphy, Tytler, and Lange on the neoplasms of the domestic fowl has aroused some interest in the frequency of their occurrence. In the course of 10 months Rous, Murphy, and Tytler[2] obtained without difficulty about 30 spontaneous tumors in living fowls. On examining 4,000 hens brought to a hotel, Ehrenreich[3] found 7 malignant tumors. All of these occurred in hens more than 1 year old, of which there were 1,000.

For the last 8 years it has been the routine practice at the Maine Agricultural Experiment Station to make autopsies on all birds that either die from natural causes or are killed by accident or for data. In making these autopsies it has been the uniform practice to record the presence of tumors, the organs in which they occur, and whether or not the tumor is of cystic or solid tissue structure. No further study has been made of any tumor. The data were collected primarily because of the possible effect of the presence of the tumor on the other data taken. In going over the records lately, however, their bearing on the frequency of the occurrence of neoplasms in fowls has seemed worthy of analysis and publication. The archives of the laboratory now contain 880 autopsy records sufficiently complete for use in this study.

Of the 880 birds on which autopsies were performed carefully, 79, or 8.98 per cent, had tumors of one sort or another. If we may consider these 880 birds a random sample of fowls as a whole, we may conclude that there are about 90 cases of tumors per 1,000 fowls. While these fowls are not a fair random sample, they are probably nearer one than any other equally large group on which data are at present available. It is possible, however, by the analysis of these records to study the frequency of occurrence of tumors in birds that die from natural causes compared to the frequency in normal birds that are killed. It is also possible to study the relation of the occurrence of tumors to age and sex.

It is a well-known fact that in man there are many tumors which do not primarily affect the health of the host. This seems to be equally true of fowls. Table I shows the occurrence of tumors, first, in birds that

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 86.

[2] Rous, Peyton, Murphy, J. B., and Tytler, W. H. A filterable agent the cause of a second chicken-tumor, an osteochondrosarcoma. *In* Jour. Amer. Med. Assoc., v. 59, no. 20, p. 1793-1794. 1912.

[3] Ehrenreich, M., and Michaelis, L. Ueber Tumoren bei Hühnern. *In* Ztschr. Krebsforsch., Bd. 4, Heft 3, p. 586-591. 1906.
Ehrenreich, M. Weitere Mitteilungen über das Vorkommen maligner Tumoren bei Hühnern. *In* Med. Klin., Jahrg. 3, No. 21, p. 614-615. 1907.

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either died from or were killed because of disease, and, second, in apparently normal birds accidentally killed or killed for data.

TABLE I.—*Percentage of tumors found in birds dead from natural causes and in normal birds which were killed*

Manner of death.	Total number of birds.	Percentage of birds with tumors present.
Natural causes	660	8.94
Killed	220	9.09
Total	880	8.98

This table shows that there was no significant difference in percentage of tumors found between the two groups of birds. Some of the tumors found in the apparently normal birds were probably early stages of tumors which might later have caused the death of the individual affected. A study of the individual cases of birds with tumors (see Table IV) shows that while in several cases the tumors were the probable cause of death, yet there were many others among the birds which died from natural causes in which the cause of death was entirely unrelated to the presence of the tumor. The close agreement of the two groups in percentage of birds with tumors strengthens the conclusion that in this flock at least there are about 90 cases of tumors per 1,000 birds.

In order to study the influence of age and sex upon the occurrence of tumors, age-frequency distributions were made for each sex. The birds were grouped into half-year classes. There were a few birds whose exact age was not known. These could be classified as "young" (under 2 years) or "old" (over 2 years). The percentage of the birds of each age group which had tumors was then calculated separately for each sex and for the two sexes together. These data are given in Table II.

This table shows that of the 880 birds only 44 were males, while 836 were females. This difference is due merely to the fact that in the adult flocks only a few males were kept (for breeding purposes) and a great many females. It indicates nothing as to the relative morbidity of males and females. Considering the small number of males, it is possible that the apparent difference in the sexes in regard to the occurrence of tumors, 6.82 per cent in the males and 9.09 per cent in the females, may not be significant. A study of the individual cases, however (see Table IV), shows that the organs most frequently affected in the females are the genital organs. It may easily be that on this account there is a real difference in the sexes.

A study of Table II shows that there is a significant correlation between age and the percentage of birds which have tumors. This is also shown in Table III, which is a summary of the data in Table II, combining the

data on all the birds, whether or not their exact ages were known, into two classes, young (under 2¼ years) and old (over 2¼ years).

TABLE II.—*Relation of age and sex to the occurrence of tumors in the domestic fowl*

Age in years (mid-points of class).	Females.				Males.				Males and females.			
	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.
½	5	81	86	5.81	0	4	4	0	5	85	90	5.56
1	39	424	463	8.42	1	24	25	4.00	40	448	488	8.20
1½	5	105	110	4.55	1	3	4	25.00	6	108	114	5.26
2	4	60	64	6.25	0	1	1	0	4	61	65	6.15
Total, ¼ to 2¼ years	53	670	723	7.33	2	32	34	5.88	55	702	757	7.27
2½	3	22	25	12.00	0	0	0	.....	3	22	25	12.00
3	5	18	23	21.74	0	1	1	0	5	19	24	20.83
3½	1	1	2	50.00	0	0	0	.....	1	1	2	50.00
4	0	1	1	0	0	1	1	0	0	2	2	0
4½	1	0	1	100.00	0	0	0	.....	1	0	1	100.00
5	0	2	2	0	0	0	0	.....	0	2	2	0
5½	0	0	0	.....	0	1	1	0	0	1	1	0
6	1	0	1	100.00	0	1	1	0	1	1	2	50.00
Total, 2¾ to 6½ years	11	44	55	20.00	0	4	4	0	11	48	59	18.64
Total, ¼ to 6½ years	64	714	778	8.23	2	36	38	5.26	66	750	816	8.09
Exact age unknown:												
Young	1	0	1	100.00	0	2	2	0	1	2	3	33.33
Old	11	46	57	19.30	1	3	4	25.00	12	49	61	19.67
Total	76	760	836	9.09	3	41	44	6.82	79	801	880	8.98

TABLE III.—*Summary of the data showing the relation of age and sex to the occurrence of tumors in the domestic fowl*

Age.	Females.		Males.		Males and females.	
	Total number.	Percentage with tumors.	Total number.	Percentage with tumors.	Total number.	Percentage with tumors.
Young (¼ to 2¼ years)	724	7.46	38	5.56	760	7.37
Old (2¼ to 6½ years)	112	19.64	8	12.50	120	19.17
Total	836	9.09	44	6.82	880	8.98

This table shows that while only 7.46 per cent of the females under 2¼ years have tumors, 19.64 per cent of those over 2¼ years are affected. The result for the males agrees essentially with that for the females, but the number of males is too small to allow us to consider this result as necessarily significant. It is, however, quite certain that the probability of the presence of a tumor in a bird increases as the bird grows older.

The records available for this study show in which organs the tumor is located and whether it is of cystic or solid-tissue structure. These data are given in Table IV.

TABLE IV.—*Data on all the cases of tumors which have been observed at the poultry plant of the Maine Experiment Station, giving their structure and the organs in which they were located*

Autopsy No.	Bird No.	Age.	Sex.	Kind of tumor.	Tumors[a] located in or attached to— Ovary.	Oviduct wall.	Oviduct ligament.	Mesentery.	Abdominal wall.	Intestine wall.	Kidney.	Gizzard.	Liver.	Spleen.	Pancreas.	Heart.	Testis.	Breast bone.	Organ not recorded.	Cause of death.
		Yr. m.																		
491	408G	0 6	♀	Cystic															+	Unknown.
500	466G	7	♀	Tissue					+											Internal bleeding; point not recorded.
504	559G	7	♀	do					+				*	*						Probably abnormal condition of liver and spleen.
12	81D	8	♀	do											+					Killed because collapsed trachea almost prevented breathing.
967	9070	8	♀	Cystic		+														Killed for data.
193	365E	9	♀	Tissue					+											Egg bound; anal opening closed by egg.
199	222E	9	♀	do					+		*		*	*						Probably tumors.
184	656D	9	♂	do					+								+			Unknown.
222	104E	10	♀	do				+	+					+						Probably tumors.
200	321E	10	♀	do					+	+										Unknown.
109	440D	10	♀	do	+															Digestive disorders.
107		10	♀	do	+															Internal hemorrhage; point not recorded.
344	184F	10	♀	do					+		+		*							Probably tumors.
110	201D	10	♀	do	+(small)															Choked to death.
219	430E	11	♀	do							+		*							Probably tumors.
130	292D	11	♀	do	+						*		*							Internal hemorrhage; point not recorded.
117	260D	11	♀	do	+															Unknown.
147	380D	1 0	♀	Cystic									+							Probably tumors.
143	708D	1 0	♀	Tissue	+	+					*		*							Internal bleeding from tumor on liver.
138	680D	1 0	♀	do	+								*							Possibly tumors.
132	330D	1 0	♀	do	+						*									Do.
127	827D	1 0	♀	do	+(small)															Killed because she was sick. Apparently normal except tumor.
1011	117M	1 0	♀	do			+													Killed because of roup.
1014	371M	1 0	♀	do					+											Catarrh.
869	482K	1 0	♀	Cystic		+														Unknown.

258	341E	1	0	♀	...do					+										Heart and veins filled with clotted blood.
238	75E	1	0	♀	Tissue					+			*		*	*				Do.
240	148G	1	0	♀	Cystic	+							+			+				Probably tumors.
257	457E	1	0	♀	Tissue												+			Hung in trap nest.
260	497E	1	0	♀	Cystic			+												Killed for data.
389	280F	1	0	♀	Tissue									+						Do.
158	521D	1	1	♀	...do		+								*	*				Internal hemorrhage from ruptured spleen.
154	374D	1	1	♀	...do		+								*					Unknown.
152	583D	1	1	♀	...do		+													Internal hemorrhage; point not recorded.
655	87H	1	1	♀	Tissue containing cysts		+								*					Internal hemorrhage from tumor.
538	16G	1	1	♀	Tissue				+											Unknown.
536	279G	1	1	♀	...do															Probably tumors.
423	1229	1	1	♀	Cystic			+	+				+		+					Gout.
287	22E	1	1	♀	Tissue containing cysts	+														Internal hemorrhage from tumor.
255	306E	1	1	♀	Cystic	+														Peritonitis. Heart and veins filled with clotted blood.
248	429E	1	1	♀	Tissue								*			*		+		Internal hemorrhage; point not recorded.
235	432E	1	1	♀	...do						+				*	*				Heart and veins filled with clotted blood.
144	88D	1	1	♀	...do												•			Digestive disorders.
164	678D	1	2	♀	...do	+									*				+	Probably tumors.
168	652D	1	2	♀	...do		+													Digestive disorders. Toxemia.
174	61D	1	3	♀	...do	+					+									Probably tumors.
1047	72M	1	3	♀	...do				+											Died on nest with broken egg partly out.
923	646	1	4	♂	...do															Probably tumor.
331	1367 '08 ch	1	5	♀	Cystic	+									*	*		+		Internal hemorrhage from liver.
939	30K	1	6	♀	Tissue				+											Digestive disorders.
616	379H	1	7	♀	...do					+	+									Killed because of roup.
508	1233	1	10	♀	...do	+														Internal hemorrhage from tumor.
350	1133	1	11	♀	...do		+													Peritonitis due to eggs in body cavity.
60	678C	1	11	♀	Cystic								+		*					Killed for data.
1017	1548	2	0	♀	Tissue	+			+		+									Probably tumors.
307	1083	2	3	♀	...do			+	+	+	+									Do.
713	1280	2	5	♀	Cystic			+												Killed for data.
810	14H	2	5	♀	Tissue	+														Killed for meat.
69	521B	2	11	♀	...do	+									*					Killed for data.
84	99B	2	11	♀	...do	+														Do.

[a] Asterisk (*), organ hypertrophied probably by infiltration with tumor cells.

TABLE IV.—*Data on all the cases of tumors which have been observed at the poultry plant of the Maine Experiment Station, giving their structure and the organs in which they were located*—Continued

Autopsy No.	Bird No.	Age.	Sex.	Kind of tumor.	Tumors[a] located in or attached to—															Cause of death.
					Ovary.	Oviduct wall.	Oviduct ligament.	Mesentery.	Abdominal wall.	Intestine wall.	Kidney.	Gizzard.	Liver.	Spleen.	Pancreas.	Heart.	Testis.	Breast bone.	Organ not recorded.	
		Yr. m.																		
83	130B	2 11	♀	Tissue	+															Killed for data.
414	1074	3 0	♀	do			+													Congestion of lungs.
296	1067	3 1	♀	do		+														Do.
908	20H	3 5	♀	Cystic	+					+										Roup.
952	1229	4 7	♀	do	+					+										Killed because of roup.
960	9024	5 10	♀	do	+															Peritonitis due to egg masses in body cavity.
973	000	Young.	♀	do		+														Unknown.
185	13A	Old.	♀	Tissue	+															Do.
181	8A	Old.	♀	do	+															Do.
81	187	Old.	♀	do				+												Killed for data.
45	349	Old.	♀	do	+			+												Do.
89		Old.	♀	do	+								***							Do.
68	395	Old.	♀	do	+								***							Do.
58	50	Old.	♀	do	+															Do.
80	139	Old.	♀	Cystic	+															Do.
65	152	Old.	♀	do	+															Do.
94	2	Old.	♀	Tissue	+															Do.
93		Old.	♀	do	+															Do.
954		Old.	♂	do														+		Probably tumor.
Total	79	-	{76 ♀ 3 ♂}		37	10	8	8	13	5	5	1	2	2	2	1	1	1	2	98 tumors.
Total percentage[b]					37.76	10.20	8.16	8.16	13.27	5.10	5.10	1.02	2.04	2.04	2.04	1.02	1.02	1.02	2.04	

[a] Asterisk (*), organ hypertrophied probably by infiltration with tumor cells.

[b] Percentages are calculated on base of 98 tumors, although they all occurred in 79 birds.

Attention has already been called to the fact that tumors occurred as frequently in apparently normal birds which were killed as in those which died from natural causes. From the data given in Table IV it may be seen that many of the birds with tumors died from diseased conditions apparently not related to the presence of the tumors. There were, however, a number of cases where the size and distribution of the tumors and the condition of the organs to which they were attached indicated that the tumors were the probable cause of death. Associated with many cases of tumors was a hypertrophied condition of the liver, spleen, or kidneys. The liver was most often affected. In fact, 19, or 24.05 per cent, of the individuals having tumors had enlarged and soft, friable livers. In the absence of microscopic examination of these organs, it can not be definitely stated that this hypertrophy was due to infiltration with tumor cells.

Table IV also shows that in several cases the immediate cause of death was internal hemorrhage, either from the tumor surface, the tissue immediately beneath, or the hypertrophied liver or spleen. There were several tumor cases in which death was recorded as due to internal hemorrhage but in which the bleeding point was not recorded. It is probable that in these cases also the bleeding took place either from the tumor or from the hypertrophied liver or spleen.

Our macroscopic examination of the tumors limited their classification to the two groups of tissue tumors, formed of solid masses of tissue or sometimes of large tissue masses inclosing masses of pus, mucus, or clotted blood, and cystic tumors, which were sacs filled with liquid. Table IV shows that 18, or 22.78 per cent, of the tumors observed were cystic, while 59, or 74.68 per cent, were tissue tumors. There were two cases (2.59 per cent) of ovarian tumors where cysts were attached to tissue tumors.

Table IV also shows the organ distribution of the tumors. It should be borne in mind that this is essentially the distribution in females, as only three males are included in the data. The organ most frequently affected is the ovary (37.76 per cent [1] of all the tumors occur in that organ). The oviduct wall and ligament harbored 18.36 per cent—that is, in the female the genital organs are the organs most frequently affected by tumors. The number and percentages for each of the other organs are given in the table. Table IV also shows that in most cases the tumor was confined to one organ. In 15 cases, however, the tumor had undergone metastasis, since tumors of similar sorts occurred in 2 (11 cases), 3 (3 cases), or 4 (1 case) organs. Attention has already been called to the frequent association of hypertrophied livers, spleens, and kidneys with defined tumors in other organs.

[1] These percentages are calculated on the basis of 98 tumors, although they all occurred in 79 individuals

SUMMARY

The purpose of the present paper is to record the data on the frequency of occurrence of tumors in the domestic fowl which have been collected during eight years' routine autopsy work at the Maine Agricultural Experiment Station.

The chief points brought out by an analysis of these data are as follows:

(1) Of the 880 birds autopsied 79, or 8.96 per cent, had tumors. That is, there were 90 cases of tumors per 1,000 birds.

(2) There was no significant difference in frequency of occurrence of tumors between birds which died from natural causes and apparently normal birds which were killed.

(3) There is a significant positive correlation between age and the occurrence of tumors. Only 7.37 per cent of the birds under 2¼ years had tumors, while neoplasms were present in 19.17 per cent of those that were over that age.

(4) In birds with tumors which died from natural causes, the tumors were directly or indirectly the probable cause of death in from one-third to one-half the cases.

(5) There was a decided tendency for the association of hypertrophied (apparently due to cell infiltration) liver, spleen, or kidney with the presence of tumors in other organs.

(6) Death often resulted from internal hemorrhage from the tumor, the underlying tissue, or the hypertrophied liver or spleen.

(7) The tumors can be classified into cystic and tissue tumors; 22.78 per cent of the tumors were of cystic and 74.68 per cent of solid-tissue structure. There were two cases of tissue tumors to which cysts were attached.

(8) In the females[1] the organs most frequently affected were the genital organs; 37.76 per cent of all the tumors being in the ovary and 18.36 per cent in the oviduct and oviduct ligament.

(9) In most cases the tumors were confined to one organ. In 15 cases, however, the tumor had evidently undergone metastasis, since tumors of similar nature occurred in from two to four organs.

[1] Autopsies were made on too few males to yield reliable data.

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